AN INVESTIGATION OF BONE HISTOLOGY AS A POTENTIAL AGE INDICATOR IN ROE DEER

JANE LOUISE RUDDLE

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Thesis submitted in fulfilment of the requirements for the PhD degree, University of London.

Institute of Archaeology,

University College London.

ABSTRACT

The histological structure of 72 modern roe deer mandibles was examined to see if those features that showed age- related development patterns in humans (Kerley, 1965), and laboratory rats (Singh & Gunberg, 1971), could be quantified and used to predict age in a species which poses particular ageing problems to zooarchaeologists. The roe deer sample consisted of 30 bucks and 42 does ranging in age from kids through to 14 years. Bone pieces, 5mm thick, were removed from the base of each right mandibular ramus and prepared for analysis in a scanning electron microscope operated in back-scattered electron detector mode. Four fields of view per section were photographed at a magnification of x44, and the numbers of secondary osteons and non-Haversian canals, together with the area of periosteal circumferential lamellae in each was recorded. Histological feature counts were regressed on age to produce equations which could be reversed and used to predict new ages for each specimen. The average accuracy and bias of the new ages were calculated. Statistical analysis revealed that circumferential lamellae area did not show any correlation with age in roe deer, but doe total and average non-Haversian canal counts showed highly significant correlations with age ($r = -0.75, P=0.00$) and doe average non-Haversian canal counts provided the most accurate age estimates (average inaccuracy = 2.50 years, average bias = - 0.04 years). However, tooth wear scores were found to have a stronger correlation with age, for bucks and does together, ($r = 0.88, P=0.00$) and a greater level of accuracy of age prediction (average inaccuracy = 1.31 years, average bias = 0.16 years). Still, in the absence of the dentition, histology-based ageing remains the only technique that can be applied to roe deer over their entire age range.

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INTRODUCTION

Many traditional ageing techniques, such as observations of tooth wear, epiphyseal fusion and counts of incremental layers in dental tissues, may be used in the study of both human and non-human mammalian skeletal remains. These techniques have many restrictions and may only be applied to specific skeletal elements, such as the teeth, or to narrow age ranges of specimens, such as juveniles. Consequently, their value to archaeologists, who do not have the luxury of selecting their study material, is limited. However, thirty years ago a new age estimation technique emerged from the world of forensic science - the quantification of histological features within cortical bone (Kerley 1965). This technique is applicable to the study of humans throughout their entire life span, and is based on the analysis of just a small fragment of cortical bone. Since the introduction of this technique, its value in ageing poorly preserved and highly fragmented human remains has been shown (Thompson & Trinkaus, 1981; Trinkaus & Thompson, 1987; Samson & Branigan, 1987), as has its application to a non-human mammalian population, that is laboratory rats (Singh & Gunberg, 1971) and there is evidence to suggest that it may be used to age other non-human mammals (Enlow & Brown, 1958, Jowsey, 1966; Enlow, 1968; Singh *et al.*, 1974). In the present paper this potential was put to the test as histological features within bone were quantified in a sample of 72 modern roe deer, ranging in age at death from kids through to 14 years, and the relationships between resulting counts and age were investigated.

Roe deer were selected for analysis as they are commonly encountered on archaeological sites dating from the Mesolithic period onwards (Legge & Rowley-Conwy, 1985), but they pose a particular problem to archaeologists, in that their permanent dentition is completed by the end of their first year of life. As a result more traditional ageing techniques, such as observations of tooth eruption, are of limited application and the accuracy of other ageing techniques, including tooth wear and cement layering, has not been investigated in animals over 8 years old. In addition, the bone development and growth rates in a modern 'wild' species were more likely to be representative of the same processes in ancient individuals (of the same species), than they would for a modern domestic species and its ancient progenitor. Wild roe deer therefore served as a better starting point for tests of the method than domestic animals. Finally, a large aged collection of roe deer was available for analysis whereas it is difficult to obtain such collections of the main domesticates.

This thesis opens with a review of the current range of traditional age estimation techniques that are applied to roe deer, and cervids in general. The particular advantages and disadvantages of each technique are reviewed, but the chapter concludes that there is a need for either the refinement of existing techniques or for the development of a new technique not so limited in its potential application. A new technique, 'borrowed' from forensic science and based upon counts of histological features within bone, is suggested as a possible alternative. In Chapter 2 the development of the microscopic structure of bone is discussed, and this provides the background knowledge to the studies discussed in Chapter 3, which have used the quantification of bone histological structures to age modern and ancient humans, and rats. In Chapter 4, the preparation for histological analysis of the roe deer sample, which formed the focus of this project, is presented.
Previous histology-based ageing techniques have relied upon the quantification of features as they appeared in thin sections of bone viewed under a polarised or transmitted light microscope. Here the preparation of thin sections of bone was found to be extremely time consuming and, although various approaches were adopted, the successful production of an intact section could not be guaranteed. Hence the project used for the first time the alternative method of scanning electron microscopy in back-scattered electron mode (SEM-BSE), which simply necessitates the production of cut, polished block-faces of bone. A camera was attached to the SEM so that photographs of the required bone fields could be made, and counts of histological features were then conducted directly from the photographs using a standard procedure. In Chapter 5 the relationships between these counts and age in roe deer are investigated, and the value of the technique as an age indicator in this species is considered. Chapter 6 concludes this report and suggests avenues for future research.

CHAPTER 1 AGE ESTIMATION

In this chapter the strengths and weaknesses of the current range of age estimation techniques are considered. As this project is concerned with an investigation of the potential of bone histology as an age indicator in roe deer, only those techniques applicable to the study of deer are included. Where necessary, references to studies of species other than deer are made to highlight the advantages or disadvantages of those age estimation techniques considered.

Recovered faunal remains can be aged by either:

1.1 Macroscopic, Comparative Techniques

1.2 Incremental Count Techniques

1.1 Macroscopic, Comparative Techniques

Certain aspects of skeletal and dental development occur in sequences which have been documented with respect to age for most domestic species and many other ungulates. Consequently recovered bones, antlers and teeth can potentially be aged by comparing their state of development with standards developed from comparative modern stock of known age. Ages assessed in this way are 'relative ages' rather than 'absolute ages', and are an expression of the state of biological development of one animal in relation to another. Developmental processes in deer that have been documented with respect to age include:

1.1.1 Epiphyseal Fusion And Bone Growth

1.1.2 Antler Growth

1.1.3 Tooth Development Within The Jaw

1.1.4 Tooth Eruption And Succession

1.1.5 Tooth Wear

These development processes are discussed below, and the advantages and disadvantages of each as a basis for age determination is given special consideration.

1.1.1 EPIPHYSEAL FUSION AND BONE GROWTH

Most post-cranial bones develop from separate centres of ossification that fuse during the juvenile period or with the onset of maturity. For example, limb bones consist of a central shaft or diaphysis and two ends or epiphyses. During growth a diaphysis is separated from its epiphyses by cartilaginous plates, but once development is complete, the plates are gradually replaced by bone and the three separate parts of each limb bone are unified to form a single unit (Chapter 2). The bony union of a diaphysis with its epiphyses is known as epiphyseal fusion, and skeletal maturity is attained when all epiphyses are fused (Silver; 1969, p. 284). Other post-cranial bones such as the pelvis, vertebrae and the scapula also develop initially from separate parts that fuse together by maturity. Different bony elements develop at differing rates and they fuse at varying ages, in a sequence that is generally similar for all individuals of the same species. The timing of the sequence is established by examining modern animals of known age. Ideally, the state of development is examined in cleaned skeletons or individual bones, but fusion is a gradual process that may take months to be completed. In juvenile specimens, where fusion has barely begun, cleaning of fresh bone specimens results in destruction of the soft cartilaginous epiphyseal plate and bony epiphyses become detached, whereas the fused epiphyses do not separate in this way. There are, however, intermediate stages in which epiphyses and diaphyses are joined by fragile bone bridges than can fracture easily whilst cleaning. In specimenswherefusion occurred recently,a fusion line marking the join of a bone shaft and an epiphysis can be seen, whilst in the most mature specimens remodelling results in the obliteration of the fusion line. Fusion in live animals can also be studied by using x-rays where epiphyseal cartilage plates are less dense than bone, and so show up as dark bands (Morris, 1972). Different stages of union are represented by varying prominence of this band. To age archaeological specimens using epiphyseal fusion, the degree of fusion is assessed by eye and the state of development compared with the standard tables based on modern deer (below).

Fusion data for cervids is limited and most studies have been confined to the examination of the lower limb extremities (metapodials and phalanges), as these bones are easily removed in the field and are of no meat value (Knight, 1966; below), however , the sequence and timing of fusion has been detailed for:

- Black-Tailed Deer (*Odocoileus hemionus*)
- 'American' Elk (*Cervus canadensis*)
- Roe Deer (*Capreolus capreolus*), Fallow Deer (*Dama dama*) and Red Deer (*Cervus elaphus*)
- 'European' Elk (*Alces alces*)
- White-Tailed Deer (*Odocoileus virginianus*)

- **Black-Tailed Deer (*Odocoileus hemionus*)**

Lewall & Cowan (1963) studied fusion in the limb bones of 24 male and 10 female culled, pen-reared, animals of known age. Three males and one female had been reared on a restricted diet, and had only been given 70% of the food given to each of the other animals. Bones were cleaned prior to examination and the degree of fusion attained was assessed through x-ray analysis. Four stages of fusion were noted:

A) Open - the epiphysis separated from the diaphysis during preparation.

B) Broad closure - a broad dark shadow could be seen between the epiphysis and diaphysis

C) Thin - a thin dark shadow could be seen between the epiphysis and the diaphysis.

D) Obscured - no line or shadow could be seen between the epiphysis and diaphysis.

Fourteen epiphyses (Table 1.1) were scored from 0 (open) to 3 (obscured) in each animal and, thus, all had scores of between 0 (no bones fused) to 42 (all bones fused). It was noted that, in general:

- Fore limbs fused before hind limbs.
- The proximal tibia and humerus, and distal radius fused 12 months later in females than males.
- The distal femur fused 18 months later in males than females.
- Although the sequence of fusion was different for males and females, both showed complete fusion by 78 months.
- The bones of animals raised on a restricted diet fused 12 months later than those fed a full diet.

Table 1.1: Ages At Which Fourteen Epiphyses Reached Stage B (Broad Closure) In Black-Tailed Deer (after Lewall & Cowan, 1963)

Ages in Months					
Element	**Male**	**Female**	**Element**	**Male**	**Female**
Radius, proximal	14	14	Femur, proximal	34	29
Humerus, distal	14	17	Tibia, proximal	34	35 to 60
Phalanges	14	14	Ulna, proximal	34	35
Tibia, distal	15	14	Ulna, distal	52	35 to 60
Calcaneum	27	29 to 35	Radius, distal	34	35 to 60
Metatarsus	29	29 to 35	Humerus, proximal	52	35 to 60
Metacarpus	29	29	Femur, distal	52	35

Table 1.2: Ages By Which The Distal Radius And Distal Ulna Fused In 'American' Elk (after Knight, 1966)

Ages are in years		
Element	**Females**	**Males**
Radius, distal	2 to 3	3
Ulna, distal	2 to 3	2 to 3

Table 1.3: Ages Of Phalangeal And Metapodial Fusion In Roe Deer, Red Deer And Fallow Deer (after Bosold, 1968 cited in Iregen, 1975)

Species & Element	Age in Months
Roe Deer Phalanx II	6 to 7
Roe Deer Phalanx I	8 to 10
Red and Fallow Deer Phalanges	12 to 15
Roe Deer Metapodials (metacarpals before metatarsals)	24 to 30
Red and Fallow Deer Metapodials	30

Table 1.4: Ages Of Fusion In The Lower Limbs Of 'European' Elk (after Iregen, 1975)

Element	Age in Years	Element	Age in Years
Phalanx II	calves to 2	Metapodials	1 to 2
Phalanx I - fore limb	calves to 1	Radius, distal	2 to 3
Phalanx I - hind limb	1 to 2	Ulna, distal	3 to 4

Table 1.5: Fusion Ages In White-Tailed Deer (after Purdue, 1983)

	Ages in months					
	Initial Fusion		Half-fused		Complete Fusion	
Element	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Radius, proximal	1 to 3	4 to 6	1 to 3	4 to 6	4 to 6	7 to 9
Humerus, distal	1 to 3	4 to 6	1 to 3	7 to 9	19 to 21	N/A
Phalanx II	4 to 6	7 to 9	4 to 6	7 to 9	by 12	10 to 12
Phalanx I	by 12	10 to 12	by 12	10 to 12	19 to 21	16 to 18
Tibia, distal	by 18	by 18	by 18	16 to 18	19 to 21	22 to 24
Calcaneum	by 18	19 to 21	19 to 21	19 to 21	by 30	by 30
Ulna, proximal	19 to 21	19 to 21	19 to 21	19 to 21	by 30	by 40+
Femur, proximal	19 to 21	19 to 21	19 to 21	19 to 21	21 to 33	by 40+
Metacarpal	19 to 21	22 to 24	19 to 21	22 to 24	by 30	by 30
Metatarsal	19 to 21	16 to 18	19 to 21	22 to 24	by 30	by 30
Radius, distal	19 to 21	by 30	19 to 21	by 30	by 30	by 40+
Femur, distal	22 to 24	by 30	22 to 24	by 30	by 30	by 40+
Tibia, proximal	22 to 24	by 30	22 to 24	by 30	by 30	by 40+
Ulna, distal	22 to 24	by 30	by 30	by 30	by 30	by 36
Humerus, proximal	by 30	by 40+	by 30	by 40+	40+	by 40+
Cervical vertebra, centrum	1 to 3	1 to 3	1 to 3	1 to 3	4 to 6	4 to 6
Thoracic vertebra, centrum	1 to 3	1 to 3	1 to 3	1 to 3	4 to 6	4 to 6
Lumbar vertebra, centrum	1 to 3	1 to 3	1 to 3	1 to 3	4 to 6	4 to 6
Acetabulum	4 to 6	7 to 9	4 to 6	7 to 9	by 12	10 to 12
Atlas, dorsal	by 12	7 to 9	by 12	7 to 9	by 18	19 to 21
Sacrum	by 18	10 to 12	by 18	16 to 18	40+	N/A
Lumbar vertebra, posterior	22 to 24	16 to 18	22 to 24	19 to 21	34 to 36	40+
Lumbar vertebra, anterior	19 to 21	16 to 18	by 30	19 to 21	34 to 36	40+
Thoracic vertebra, posterior	by 30	N/A	by 30	N/A	40+	N/A
Thoracic vertebra, anterior	22 to 24	by 30	by 30	by 30	by 30	N/A
Cervical vertebra, anterior	by 30	by 36	by 30	by 36	40+	by 40+
Cervical vertebra, posterior	by 30	40+	by 30	by 40+	40+	by 40+
Pubic symphysis	22 to 24	40+	by 30	by 40+	40+	by 40+

N/A - specimens not available.

- **'American' Elk (*Cervus canadensis*)**

As part of a study of bone characteristics associated with ageing in 'American' elk (below), Knight (1966) noted the ages by which the distal radius and distal ulna fused in 30 animals from the Sun River Checking Station at Augusta, Montana and 444 animals from Yellowstone National Park. All animals were aged through the tooth replacement and wear standards of Quimby & Gaab (1957; below), and bones were cleaned of all flesh prior to examination. The distal radius-ulna was selected for analysis as it "is easily removed with a saw at checking stations, and removal does not damage edible portions" (Knight, 1966, p. 369). Several observations were made (Table 1.2):

- In females the distal radius was unfused in all calves, yearlings and in 45% of all 2 year olds. It was fused in all > 2 years old.
- In males the distal radius was unfused in all calves, yearlings and 2 year olds. It was fused in both 3 year olds, in six out of nine 4 year olds and in all > 4 years old.
- In females the distal ulna was unfused in all calves and yearlings, in 88% of 2 year olds and 9% of 3 year olds. It was closed in all > 3 years old.
- In males the distal radius was unfused in all calves and yearlings and in four out of nine 2 year olds. It was fused in both 3 year olds and all > 4 years.

Fusion data was also combined with observations of the medial tuberosity, the vascular groove and the volar central surface of the distal radius to produce a key for age determination in elk, up to 4 years:

Knight's (1966) Key to Age Determination in Elk

1a Epiphyseal cartilage of radius open - **go to 2**

1b Epiphyseal cartilage of radius closed - **go to 5**

2a Medial tuberosity present - **go to 3**

2b Medial tuberosity absent or poorly formed - **calf**

3a Medial tuberosity present as a thin layer with finely feathered edges - **yearling**

3b Medial tuberosity thickened, often with sharp edges on side nearest epipyseal cartilage - **go to 4**

4a Male - **go to 6**

4b Female - **2 years old**

5a Male - **go to 6**

5b Female - **go to 8**

6a Vascular groove incomplete - **2 years old**

6b Vascular groove incomplete - **go to 7**

7a Epiphyseal cartilage of radius open - **3 to 4 years old**

7b Epiphyseal cartilage of radius closed - **4 years or older**

8a Epiphyseal cartilage of ulna closed - **go to 9**

8b Epiphyseal cartilage of ulna open - **2 to 3 years** (88% of cases examined 2 years)

9a Ossification complete on volar central radius - **4 years** (86% of cases examined)

9b Ossification incomplete on volar central radius - **3 years** (but 15% of cases examined 4 years)

- **Roe Deer (*Capreolus capreolus*), Fallow Deer (*Dama dama*) and Red Deer (*Cervus elaphus*)**

Currently, the only study of fusion in these three deer species would seem to be that of Bosold (1968, cited in Iregen, 1975). In animals from Munich, Berne, Basel and Zurich, he noted that:

- In roe deer the second phalanx fused between 6 and 7 months, the first phalanx at 8 to 10 months and the metapodials (metacarpals before metatarsals) between 24 and 30 months (Table 1.3).
- In red deer and fallow deer all phalanges fused between 12 and 15 months and the all metapodials by 30 months (Table 1.3).

- **'European' Elk (*Alces alces*)**

Iregen (1975) noted fusion timings in the lower fore and hind limbs of 100 elk, culled within legal limits in central Sweden (Table 1.4):

- Phalanx II: This was unfused in most calves, although some did show fusion in progress. In all yearlings fusion was complete or in progress, and it was complete in all animals aged over 2 years.
- Phalanx I: In calves this was unfused in the hind legs, but fusion had begun in their fore legs. In most yearlings and all those aged 2 years or more fusion was complete.
- Metapodials: Fusion occurred between 1 and 2 years.

- Radius, distal: Fusion occurred between 2 and 3 years.
- Ulna, distal: Fusion occurred between 3 and 4 years.

- **White-Tailed Deer (*Odocoileus virginianus*)**

Purdue (1983), apparently, conducted the most comprehensive study of age-related fusion in deer. He had access to 152 complete white-tailed deer skeletons, from various museum collections in Illinois, and to the lower limbs of an additional 383 animals, from various checking stations in Illinois, Missouri and South Carolina. Bones were cleaned by the 'Biz' (an enzyme) maceration technique (Chapter 4), and most animals were aged following the tooth eruption and wear standards of Taber (1971; below). Twenty-eight epiphyses were examined on each complete skeleton, and for the partial skeletons observations were confined to the distal ends of the radius, ulna and tibia, the metapodials and the phalanges (Table 1.5). Purdue found that:

- Earlier fusing epiphyses had lower levels of individual variation than later fusing epiphyses.
- Epiphyses of female animals tended to fuse before those of males, and later fusing epiphyses showed more pronounced sexual differences.
- Geographic location did not influence fusion rates.

- **Summary of Cervid Fusion Data**

- All seven deer species detailed above show approximately the same sequence of fusion in the lower limbs: phalanx II, phalanx I and metapodials (in roe deer the metacarpal closes before the metatarsal).
- Apart from red deer and fallow deer, for which the data are very limited, the bones of different species close at different times.
- Sexual dimorphism and nutritional status appear to influence fusion timings.

In addition to the growth in length of long bones of which epiphyseal fusion forms a part, there is growth in width of bones both before and after fusion, although this is little understood, especially for roe deer. Most of the standard measurements of bones increase in size in many other mammals however, and distributions of measurements from archaeological mammals which appear to show several modes have been interpreted as seasonal occurrences where animals were hunted at intervals from their assumed birth dates (Legge & Rowley Conwy, 1985). Iregen (1975) reported that the length of the metacarpus and metatarsus could be used to separate modern calf and yearling elk (*Alces alces*) (Table 1.6).

Table 1.6: Comparative Lengths of Metacarpus and Metatarsus in Elk (after Iregen, 1975).

Element	Male Calves	Female Calves	Yearling Males	Yearling Females
Metacarpus	258 ±6.7mm	251 ±6.2mm	291 ±8.2mm	279 ±9.0mm
Metatarsus	299 ±6.9mm	343 ±2.2mm	N/A	N/A

N/A - specimens not available

1.1.1.1 The Advantages

- The degree of fusion exhibited by an archeological specimen can be quickly and easily assessed by eye, both in the field and in the laboratory and no preparation is required. Similarly, measurements can be made rapidly, using well defined standards (Driesch, 1976).
- Most post-cranial bones are comprised of at least two separate elements which fuse at some time during life so, providing comparative age related data exists, all recovered skeletal elements showing areas of fusion have the potential to be aged.
- On sites where many bones of the same species are encountered, such as at Franchthi Cave (Payne, 1975), they can be sorted into types (tibia, femur etc.) and arranged in ascending order of the level of fusion attained. This enables a rapid assessment of the overall age composition of an assemblage, and the adult to juvenile ratio can be calculated.

1.1.1.2 The Disadvantages

- Once fusion has occurred, this aspect of development provides no further information about age. However, Silver (1969; p. 284) also described age associated changes in the appearance of long bones once fusion had taken place: 'In young adults limb bones shafts are relatively long and slender and their epiphyses are large. There are few surface marks on the bone and prominences for tendon and muscle attachments are small. In the mid-adult phase bones appear more rugged and their epiphyses are relatively narrower. Small prominences associated with muscle attachment appear and depressions at the muscle origins are deep. Bones are heavy and their cortex is thick. In the oldest adults excessive resorption results in bone thinning and cortices are less dense and have large central medullary cavities.' Silver's descriptions do allow bones to be seriated based on an assessment of their appearance after fusion has taken place, although only rudimentary statements about relative age may be made. Currently, data for roe deer and other deer species are limited, in any case, and only exist for the lower limb bones.

- Age estimations are based on the assumption that fusion rates in modern, and usually highly selected, populations are similar to those in ancient stock. However, fusion timings vary between modern individuals and populations of the same species and these variations have been attributed to sexual dimorphism, nutritional status (Silver, 1969), breed and genotype (Chaplin, 1971). For example, Purdue (1983) found that the lower limb bones of female white-tailed deer fused before those of males, and Lewall & Cowan (1963) found that fusion was delayed in black-tailed deer raised on a low level nutrition diet. Modern fusion data should thus be regarded with caution when estimating the age of ancient stock (Chaplin, 1971).
- Fusion data have, in the past, been collated through the analysis of animals which have been aged through the application of another age determination techniques, such as through assessments of tooth wear (below). Consequently, the assigned fusion ages may reflect errors inherent in the original technique. For example, Purdue (1983) detailed fusion ages in a sample of sixty-seven white-tailed deer aged through assessments of tooth eruption and wear, the rates of which also vary between individuals and populations of the same species (Chaplin & White, 1969; Hillson, 1986).
- Radiographic assessment can place epiphyseal closure earlier than gross visual inspection. This is because fusion is identified in x-rays as the replacement of all epiphyseal cartilage with bone. However, such a recently formed bony link may not be strong enough to withstand the rigours of cleaning and laboratory processing, nor to support the bone if it is picked up by its epiphyses - which is a definition of fusion often employed by archaeologists.

- Fused bones are larger and more easily seen during excavation. For example, Payne (1975) conducted a survey of recovery bias at a site in northern Greece, and found that 'poor retrieval methods' biased recovery of bones to fused against unfused. In several areas the ground was excavated in the 'normal way'- hand excavation without sieving - and the waste earth was retained and wet-sieved through a 3mm mesh. The residue retained by the sieve was dried and sorted to recover finds that had been missed during initial excavation. For sheep and goats, mammals similar in size and stature to roe deer, it was found that the ratio of fused to unfused scapulae, distal humeri and proximal radii was 20:1 when recovery was conducted through normal hand excavation, but 8:1 when hand excavation was combined with wet sieving. Payne (1975) concluded that unfused bones stood a poorer chance of recovery through hand excavation as they were smaller than fused bones and were more fragile and so broke up more easily, and that hand excavation biased recovery to larger fragments of the skeleton. Maltby (1979) also noted that earlier fusing bones stood a better chance of survival and recovery than later fusing bones. He attributed this to the fact that unfused bones were more porous and less dense than fused bones and that fragments containing higher proportions of cancellous bone had less chance of survival than those which had higher proportions of cortical bone.

1.1.1.3 In Summary

- Most post-cranial bones have at least one epiphysis which closes at sometime before maturity.
- Fusion has been widely studied in many modern domestic species but data for roe deer, and other deer species, is limited.
- Preservation permitting, fusion can be easily studied by eye in archaeological bones.
- Fusion rates vary between individuals and populations of the same species.
- Fusion should only be used to suggest an age range of modern stock to which an ancient animal shows similar biological development.
- Measurements of bones, after fusion has ceased, may also allow asssessments of comparative age.

1.1.2 ANTLER GROWTH

Most male deer (but not musk and Chinese water deer), and female reindeer, grow and shed antlers each year. Successive pairs of antlers show increases in size, weight and complexity, until an animal passes its sexual peak and after this time successive pairs of antlers become less elaborate. For most species, the first pair of antlers are unbranched spikes, and more spikes or tines are added annually. Chapman & Chapman (1975) noted the development of fallow deer antlers:

- Yearlings have single spiked antlers.
- Two to three year olds have two-branched antlers.
- Those several years or older have well developed palms.

Roe deer have the simplest antlers of the common deer in Europe, consisting of straight and slender spikes which, when fully grown, fork two thirds along their length, with the posterior spike forking again to give six points per head. European roe deer develop antlers up to 30cm long, but Siberian roe deer have longer antlers, often up to 45cm (Goss, 1983). The antler pedicles begin to develop a few weeks after birth (between the end of May and beginning of July) and are fully formed by six to eight months (November to January). During this time a small button of antler also grows at the apex of the pedicle (bony elevations of the skull which form their bases), but this is shed late the following spring before the first proper set of antlers begin to grow. This first set generally consists of a pair of single spikes (full six pointer antlers may not develop until the third set) which are between a third and a half of the eventual full size. The second set are usually 75% of full size and the third set around 85% of full size. From the fourth set onwards there are no further changes in length and complexity (that is number and arrangement of tines), but the girth of both antlers and tines does increase. From the sixth set onwards, antlers start to 'deteriorate' and successive sets are shorter than preceding sets, with fewer tines. De Nahlik (1992) described a roe that had good length six point antlers at four years, but which had short tineless spikes at ten years.

Antlers are often found on archaeological sites still attached to the pedicles, which have been cut from the skull. To age a deer through an assessment of its antlers, their gross development is simply compared with that detailed for modern stock of known age. The technique should not be applied to shed antlers, as there is no way of knowing how much longer an individual lived after it shed a pair of antlers.

1.1.2.1 The Advantages

- Antler size is easily assessed by eye, with a tape or measuring board to allow measurements of length and girth, and tines may be simply counted.
- Antlers can be seriated in order of ascending development to aid rapid assessments of population age structure.
- All European male deer carry antlers during the appropriate seasons, and so are open to assessment through this age determination technique.
- In prehistory antler proved to be a useful resource because prolonged soaking in water renders it easy to carve, consequently worked antler is often encountered on archaeological sites (Hodges, 1976).

1.1.2.2 The Disadvantages

- Antler growth is highly influenced by diet, health and inherited factors, and consequently there is great variation in development between individuals of the same age. Chaplin & White (1969) noted great individual variation in antler size and complexity in fallow deer over 2 years old. Also, Chaplin (1971; p. 89) noted that "numerous deformities can occur in the antlers owing to mechanical damage during growth" and that "the quality of a particular year's growth will be strongly influenced by the condition of the beast which is a reflection of the quality of the feed that has been available both before and after antler growth is resumed."
- Only bucks (except in reindeer) carry antlers, so the ages of does cannot be determined in this way.
- Antler development is seasonal, and for that portion of the year when they are absent or still growing this method of age determination is redundant.
- Shed antlers do not allow the age at death of the deer that carried them to be determined.

1.1.2.3 In Summary

- All male deer and female reindeer, over one year old, carry fully developed antlers for approximately six months of each year.
- Generally, the size and complexity of successive sets of antlers increases with age, but in roe deer after six years successive sets become less well developed.
- Antler growth is influenced by diet, health and inherited factors and great variation is shown between individuals of the same age and species. Consequently, the appearance of the antlers should only be used to suggest a broad age category, such as juvenile, 'young' adult or 'old' adult, to which a deer belongs.

1.1.3 TOOTH DEVELOPMENT WITHIN THE JAW

Here the term tooth development is used to refer to the stages through which a tooth passes from its initiation to form the tooth germ through to crown and root formation. Using x-rays of the mandible and/or maxilla these stages can be observed and documented with respect to age. Hillson (1986) provided an extensive review of the literature on age-related tooth development in man and some other mammals, but at that time such information was not available for cervids. Since then, Brown & Chapman (1991a; 1991c) devised a method for age determination in fallow and red deer based upon observations of tooth development, and their work is reviewed below. As yet tooth development has not been detailed in roe deer.

- **Brown & Chapman (1991a; 1991c)**

Brown & Chapman (1991a) x-rayed the mandibles of fifty-six fallow deer of known age from two days to ninety-one months, and one hundred and thirteen red deer of known age from one to one hundred and thirty-eight months (Brown & Chapman, 1991c). From these x-rays they identified the chronology of mandibular permanent molariform (premolars and molars) development in fallow and red deer. Ten stages of development were identified as follows:

1) **Evidence of a Crypt:** The crypt is a resorbed hollow within the jawbone in which a tooth germ develops and grows.

Table 1.7: The Ages (In Months) By Which Five Selected Stages Of Development Were Observed In Fallow Deer (after Brown & Chapman, 1991a)

	Stage 1	Stage 2	Stage 5	Stage 8	Stage 10
PM2	9	<18	<18	22	31
PM3	9	<18	<18	<26	31
PM4	9	11	<18	22	31
M1	in utero	in utero	<4	7	27
M2	<3.5	4	9	19	31
M3	9	11	<18	27	38

Table 1.8: The Ages (In Months) By Which Five Selected Stages Of Development Were Observed In Red Deer (after Brown & Chapman, 1991c)

	Stage 1	Stage 2	Stage 5	Stage 8	Stage 10
PM2	11	<14	18	27	41
PM3	11	14	18	27	40
PM4	11	14	18	27	33
M1	in utero	in utero	4	9	28
M2	<4	4	9	18	33
M3	10	13	26	28	40

<u>Key To Tables 1.7 And 1.8</u>

PM2	Second premolar	**Stage 1**	Evidence of a crypt
PM3	Third premolar	**Stage 2**	Evidence of tooth crown mineralisation
PM4	Fourth premolar	**Stage 5**	Crown formation complete
M1	First molar	**Stage 8**	Late root formation
M2	Second molar	**Stage 10**	Root apex closed
M3	Third molar		

2) **Evidence of Mineralisation:** Mineralisation of dentine and enamel is a gradual process, which begins in the mesial cusps of the molariform teeth.

3) **All Cusps Mineralising:** The distal cusps begin to mineralise shortly after the mesial cusps.

4) **Infundibulum Formation:** As the lingual and buccal cusps develop they become separated by a deep crevice called the infundibulum.

5) **Crown Formation Complete:** Once the crown has completely developed the roots begin to grow.

6) **Early Root Formation:** Mesial and distal roots can be distinguished.

7) **Half Root Formed:** Half of the full root length has formed.

8) **Late Root Formation:** More than half the full root length has formed.

9) **Full Root Formed but Root Apex Open:** For a while after development the root apex (through which blood and nerves pass into the tooth) remains wide open, but it slowly narrows and closes over.

10) **Root Apex Closed:** The root apex finally closes over and is covered by a layer of mineralised cement.

Individual permanent premolars and molars, as seen in the x-rays, were then assigned points depending on which stage of development they had reached (those in stage one were given one point, those in stage two were given two points and so on through to stage ten), and the total development score for each mandible was calculated. This allowed them to draw up tables of the ages at which different stages were reached for different teeth (Tables 1.7; 1.8) and to fit a growth curve model to the total molariform scores that allowed aged to be predicted for an unknown jaw specimen.

The tooth development sequence was similar for both fallow deer and red deer, but the ages at which particular stages of development were reached were different. In both species the first molar was the first to pass through all stages of development. This began in utero and was completed in fallow deer by twenty-seven months and in red deer by twenty-eight months. In fallow deer this was followed by complete development of all the premolars and the second molar at thirty-one months, and then the third molar at thirty-eight months. In red deer, complete first molar development was followed by complete development of the fourth premolar and the second molar at thirty-three months, the third premolar and third molar at forty months and the second premolar at forty-one months.

To age a fallow or red deer through the Brown & Chapman technique its mandible could be x-rayed, and the permanent premolars and molars assigned development scores as detailed above. If all the premolars and molars are present and a total mandible score is calculated then the logistic growth curves devised by Brown & Chapman can provide 'a complete range of predicted ages for any total molariform score from nought through to sixty (Brown & Chapman, 1991a; 1991c). If only some of the premolars and/or molars are available, then the Brown & Chapman tables showing the ages at which various stages of development were noted for individual teeth can be used to enable rough estimates of age.

1.1.3.1 The Advantages

- Although not tested, Brown & Chapman (1991a, p. 378) stated that "tooth development is the least likely of the mineralising growth processes to be affected by adverse nutritional conditions" so variations in developmental rates between individuals and populations are likely to be minimal. However it is noted that variations in tooth development rates in humans have been identified and have been attributed to sexual dimorphism and genotype (Hillson, 1996).
- Using the data provided by Brown & Chapman, a rough assessment of age may be made even if only one developing molar or premolar tooth is available for analysis.
- Mandibles and their teeth are extremely resistant to destruction and are often encountered on archaeological sites (Hillson, 1986).

1.1.3.2 The Disadvantages

- For cervids, age-related tooth development data currently only exists for fallow and red deer, but there appears to be no reason why roe deer tooth development could not be studied in the same way.

- Tooth development is completed in fallow deer at 38 months, and in red deer at 40 months, and consequently this method of age determination is limited in its application to those under 4 years. In roe deer it is likely that this method of age determination would be even further limited in application, as it is known that their teeth erupt (and so develop) and come into wear (below) at an earlier age than both fallow deer and red deer.
- Tooth development can only be studied through x-ray analysis. Thus access to an x-ray machine and related film processing equipment is essential to practice the technique. Jaws could be sectioned to reveal internal tooth development, but it may be difficult to relate the discrete stages described by Brown & Chapman, as they appear in x-rays, to physical remains.
- For the most precise estimates of age the technique is dependent upon the survival of mandibular molars and premolars, so that a total mandibular score can be calculated.
- Brown and Chapman (1991a; 1991c) developed their data from the study of animal populations that were not evenly distributed throughout the entire tooth development sequence, and thus some ages are approximations. For example, fallow premolar crown formation was said to occur between 11 and 18 months, but there were no animals in this age group in the original sequence and the age was deduced using information as available from animals below 11 months and over 18 months. Similarly, red deer premolar crown formation was said to occur between 15 and 18 months, although no animals from this age group were actually available for study.

1.1.3.3 In Summary

- Teeth pass through a series of developmental stages which, using x-rays, may rapidly be documented with respect to age.
- Brown & Chapman (1991; 1991c) have detailed age-related permanent premolar and molar tooth development in jaws cut from fallow and red deer of known age, but as yet similar data is not available for roe deer.
- When using the technique to estimate age (in fallow deer or red deer) the most precise estimations of age occur when all the mandibular premolars and molars are present, even if only one tooth is available, the technique may still be used to enable a rough estimate of age.

- This age determination technique is limited in fallow deer to below thirty-eight months and in red deer to below forty months and, from what is known about their eruption sequence, it is likely to be limited to an even earlier age in roe deer as their teeth erupt (below) almost a year before those of fallow deer.

1.1.4 Tooth Eruption and Succession

For most mammalian species deciduous teeth erupt into the mouth, are lost and are replaced by permanent teeth in an ordered sequence which also may be related to age. The arrival of teeth in the mouth is much easier to investigate in the field than dental development (above). To age a fresh jaw, the teeth present in the mouth are compared with published standards (below). For archaeological specimens, a similar technique is used, although the appearance of teeth through tissues that communicate between the crypt and with the bone is different to emergence through the gum. It should be noted that to compensate for the effects of wear (below), the teeth of cervids, and other high crowned ungulates, continue to erupt into the mouth after the full crown height has been exposed and, consequently, the roots may show above the gum line. The sequence and timing of eruption and succession are known for most common deer species, including:

- Barren Ground Caribou (*Rangifer tarandus*)
- Fallow Deer (*Dama dama*)
- Moose (*Alces alces*)
- Mule Deer (*Odocoileus hemionus*)
- Red Deer (*Cervus elaphus*)
- Roe Deer (*Capreolus capreolus*)

- **Barren Ground Caribou (*Rangifer tarandus*)**

Full permanent dental eruption for caribou is achieved at around 22 months (Taber, 1971). Speiss (1979) adapted Miller's (1972; 1974) data to devise an age and season of death determination scheme for archaeologically recovered caribou/reindeer mandibles based on eruption (Table 1.9) and wear (below).

Table 1.9: Eruption Timing, And Season, Of The Cheek Teeth In Caribou/Reindeer Detailed by Speiss (1979)

Tooth and Eruption Stage	Age (in months)	Season/Month
Deciduous premolar	present at birth	mid May
First molar erupting	3-5	mid August to mid October
Second molar erupting	10-15	mid March to mid August
Third molar erupting	15-29	any time
Permanent premolars erupting	22-29	mid March to mid September

Table 1.10: The Percentage of Fallow Deer From Richmond Park In Each Age Group With Each Tooth In The Lower Jaw Erupted (after Chapman & Chapman, 1970)

Age, months	M_1	I_1	M_2	I_2	I_3	C	M_3	P_4	P_3	P_2
0-2 (n=16)	0	0	0	0	0	0	0	0	0	0
3-4 (n=4)	50	0	0	0	0	0	0	0	0	0
5-6 (n=23)	100	0	0	0	0	0	0	0	0	0
7-8 (n=17)	100	24	0	0	0	0	0	0	0	0
9-10 (n=22)	100	100	18	0	0	0	0	0	0	0
11-14 (n=8)	100	100	75	10	0	0	0	0	0	0
15-18 (n=25)	100	100	100	100	100	100	16	0	0	0
19-22 (n=23)	100	100	100	100	100	100	52	100	10	0
23-26 (n=4)	100	100	100	100	100	100	100	100	100	75
27-30 (n=38)	100	100	100	100	100	100	100	100	100	100

<u>Key</u>

M_1 - first molar
I_1 - first incisor
M_2 - second molar
I_2 - second incisor
I_3 - third incisor
C - canine
M_3 - third molar
P_4 - fourth premolar
P_3 - third premolar
P_2 - second premolar

Table 1.11: The Pattern Of Tooth Eruption And Succession Noted In Moose (after Peterson, 1955)

Age,	Incisors			C	Premolars			Molars		
months	1	2	3	1	2	3	4	1	2	3
Birth	D	D	D	D	(D)	(D)	(D)			
1 month	D	D	D	D	(D)	D	D			
3 months	D	D	D	D	D	D	D	(P)		
5 months	D	D	D	D	D	D	D	P		
9 months	P	D	D	D	D	D	D	P	(P)	
12 months	P	D	D	D	D	D	D	P	P	
14 months	P	D	D	D	D	D	D	P	P	(P)
17 months	P	P	P	(P)	(P)	(P)	(P)	P	P	(P)
19 months	P	P	P	P	P	P	P	P	P	P

<u>Key</u>

D - deciduous tooth fully erupted

P - permanent tooth fully erupted

() - in process of eruption

C - canine

Table 1.12: The Pattern Of Tooth Eruption And Succession Noted In Mule Deer (after Cowan, 1936; Dasmann, 1958; both cited in Taber, 1971)

Age	Incisors			C	Premolars			Molars		
months	1	2	3	1	2	3	4	1	2	3
<1	D	D	D	D	D	D	D	-	-	-
2 to 3	D	D	D	D	D	D	D	(P)	-	-
6	D	D	D	D	D	D	D	(P)	(P)	-
12	P*	D P	D	D	D	D	D	P	(P)	-
18	P	P	P	D	D	D	D	P	P	(P)
24	P	P	P	P	(P)	(P)	(P)	P	P	(P)
30	P	P	P	P	P	P	P	P	P	P

<u>Key</u>

***** - replacement and eruption occuring

D - deciduous tooth fully erupted

P - permanent tooth fully erupted

( ) - in process of eruption

C - canine

- **Fallow Deer (*Dama dama*)**

In a survey of both wild and park male fallow deer from lowland Britain, Chaplin & White (1969) observed permanent dental eruption (only the mandibular cheek teeth were studied) to be completed by the end of the second year of life, and the general sequence of permanent eruption was - the first molar, the second molar, the third molar and/or the fourth premolar, and the second and third premolars. Chapman & Chapman (1970) reported the eruption sequence of all the permanent teeth of fallow deer as follows:

- ♦ In the lower jaw - the first molar, the first incisor, the second molar, the second incisor, the third incisor and canine, the third molar, the fourth premolar, the third premolar and the second premolar.
- ♦ In the upper jaw - the first molar, the second molar, the third molar, the fourth premolar, the third premolar and the second premolar.

Full eruption was noted to occur in the majority of animals between 23 and 30 months (Table 1.10), a little later than as reported by Chaplin & White (1969).

- **Moose (*Alces alces*)**

Full permanent dental eruption for moose is achieved much earlier than for most other large cervids, at around 19 months (Peterson, 1955; Table 1.11).

- **Mule Deer (*Odocoileus hemionus*)**

Full permanent dental eruption for mule deer is achieved at around 30 months (Taber, 1971), and the pattern and timing of eruption has been studied by Cowan (1936) and Dasmann (1958) (both cited in Taber, 1971), and is summarised in Table 1.12.

- **Red Deer (*Cervus elaphus*)**

Red deer are born with 11 deciduous teeth - four lower incisors, an upper canine and three lower and three upper premolars (Mitchell, 1963). Deciduous tooth loss begins with the first incisor around 14 to 15 months and ends with the third premolars around 25 to 26 months. The first permanent tooth

Table 1.14: The Sequence of Mandibular Tooth Eruption and Wear as Noted in 110 Roe Deer From Thetford Chase, Norfolk (after Aitken, 1975)

Age (in years)	Tooth Eruption and Wear
< 1	All had all deciduous premolars and permanent molars.
1 to 2	All had the full adult dentition. The second and third premolars were slightly worn. A thin line of dentine was exposed on the occlusal surface of the fourth premolar. All molars had high lingual cusps. The posterior cone of M3 had recently erupted and was unworn.
2 to 3	Thin lines of dentine were exposed on the occlusal surfaces of the third and fourth premolars. All molars showed signs of wear on their buccal cusps. Molar lingual cusps were still high. The posterior cone of the third molar was slightly worn, and a small area of dentine was exposed.
3 to 4	Several specimens had dentine exposure towards the posterior surface of the second premolar, and the crest of the third molar was almost flattened. Both the buccal and lingual cusps of all molars in all specimens were reduced in height, and the posterior cone of the third molar was greatly worn on the buccal side.
4 to 5	Most specimens had a distinct area of dentine exposure on the posterior surface of the second premolar. The crest of the third molar and the lingual cusps on the anterior cone of the fourth premolar were worn flat. The dentine exposed on the posterior cone of the third molar was continuous with that on the lingual cusp of the second cone.
5 to 6	The lingual cusps of the first molar were worn flat, and to low crests on the second and third molars. The anterior buccal cusp of the first molar was also noticeably worn.
6 to 7	The occlusal surfaces of the premolars were worn flat. The lingual crests of the molars were also worn flat, and the buccal cusps were further reduced in height.
7 to 8	The first molar was so worn that the anterior infundibulum had been eroded, and the dentine of the anterior lingual and buccal cusps was continuous across the tooth. The posterior infundibulum of the first molar was reduced to a narrow slit.

to erupt is the first molar, which is function between 4 and 6 months. All permanent molars are funtional between 2½ and 3 years (Habermehl, 1961, Table 1.13; Mitchell, 1963).

Table 1.13: Eruption Ages of the Cheek Teeth in Red Deer (after Habermehl, 1961)

Tooth	Eruption Age (in months)
Deciduous premolar	2-5
Second premolar	27+
Third premolar	27+
Fourth premolar	27+
First molar	5-12
Second molar	12-24
Third molar	27+

- **Roe Deer** ***(Capreolus capreolus)***

As with other deer species, roe deer are born with their full deciduous dentition erupted, but replacement with the permanent dentition is much more rapid and is completed toward the end of their first year of life (Aitken, 1975; De Nahlik, 1992). Aitken (1975) detailed the pattern of tooth eruption and wear (below) in roe deer mandibles (Table 1.14).

1.1.4.1 The Advantages

- In roe deer the third molar is the last tooth to erupt, and marks full maturity so that its presence or absence may be used rapidly to separate juveniles and adults.
- Just as post-cranial bones may be seriated in ascending order of the level of fusion attained, jaws may be seriated in ascending order of tooth eruption and succession, again, to enable a rapid assessment of the overall age composition and/or juvenile to adult ratio of an assemblage.
- The presence or absence of teeth in the jaw can be easily recorded by eye.
- The presence of a specific tooth may be sufficient to assign a minimum or maximum age.
- Like tooth development, this method of age determination benefits from the fact that teeth and jaws are extremely resistant and make common finds on archaeological sites.

1.1.4.2 The Disadvantages

- As the eruption of all teeth into the mouth is completed by the onset of maturity, the application range of this age estimation technique is limited. In roe deer this stage is reached by twelve months (Aitken, 1975). This limitation may be overcome if observations of tooth eruption and succession are combined with observations of tooth wear (below), to provide a continuous sequence of age-related development throughout life.
- This age determination technique relies upon the survival and recovery of the bulk of the dentition or 'key' teeth, for which specific eruption and/or succession timings are known.
- Definitions of tooth eruption vary, and contemporary tooth eruption data comes from studies of live animals where gingival emergence (the cutting of the teeth through the gums) is used as the reference point for eruption. In archaeological contexts, eruption refers to the cutting of the first communication of a tooth crypt with the surface of the bone (Garn, Koski & Lewis, 1957). There is an element of delay in timing between such eruption through the bone and gingival emergence, and so care must be taken when using data derived from studies of live animals to age archaeologically recovered remains.
- Considerable variation between individuals, breeds, sexes and populations in tooth eruption and succession rates have been noted (Chapman & Chapman, 1970; Chaplin, 1971; Hillson, 1986; 1996).
- Tooth eruption and succession does not occur in evenly distributed time bands throughout the growth period of an animal, so that several 'key' teeth may all erupt within a month of each other but then no significant stages may occur for several more months.

1.1.4.3 In Summary

- Deciduous teeth erupt and are replaced by the permanent dentition in ordered, species-specific sequences.
- These sequences are relatively simple to document in recently culled deer carcasses, and so much comparative data exists for many deer species.

- The application range of this age determination technique is limited until the last tooth in an eruption sequence has come into the mouth, which in roe deer is the third molar at around twelve months.

1.1.5 TOOTH WEAR

Once teeth erupt into the mouth they start to wear. Wear is most prominent on occlusal surfaces, and it is caused by grinding of opposing teeth against each other during the chewing of food. Approximal wear may also be noted in those areas where adjacent teeth contact each other, but this is not used in age determination. In high crowned teeth, especially, wear commences before a tooth has fully erupted and continues for as long as that tooth is functional. Tooth wear can be recorded in three ways:

- Simple Visual Assessment
- Scoring
- Measuring

- **Simple Visual Assessment**

This is the oldest and most widely used method of age determination for domestic and herded, or managed, species, and gave rise to the saying "never look a gift horse in the mouth", attributed to Saint Jerome of the fifth century (Chapman & Chapman, 1970). Wear is assessed through the observation of the changing patterns and proportions of cement, enamel and dentine that are revealed on the occlusal surface of a tooth as deeper layers of its internal structure are gradually exposed. To age an unknown animal, its dentition is examined and the pattern of wear seen is compared with that detailed for comparative modern stock of known age. Lowe (1967) states that Nitsche (undated) was probably the first to detail age related tooth wear stages in deer. Since then, tooth wear patterns have been described for most cervids, including elk (*Cervus canadensis*) (Murie 1951) caribou/reindeer (*Rangifer tarandus*) (Speiss, 1979), fallow deer (*Dama dama*) (Chaplin & White, 1969; Chapman & Chapman 1970), red deer (*Cervus elaphus*) (Habermehl, 1961; Mitchell, 1963, 1967), white-tailed deer (*Odocoileus virginianus*) and roe deer (*Capreolus capreolus*) (Anderson, 1953; Aitken, 1975;

Table 1.14). However, varying levels of success in using such patterns to estimate age have been reported:

- Murie (1951) studied elk (*Cervus canadensis*), of unknown age, and found that different age classes could be clearly distinguished up to 3 years. After this the pattern of attrition became more variable - this was thought to be due to the fact that the mandibular tooth row was longer than the maxillary tooth row and, so, individuals could chew more heavily on either their back molars or anterior premolars as preferred.
- Severinghaus (1949) studied white-tailed deer (*Odocoileus virginianus*) of known age, and found that observations of tooth wear could be used to age individuals up to 10 years.

- **Scoring**

This involves scoring wear on a more detailed level by awarding points to defined wear stages for each slope on a tooth's occlusal surface. The points awarded to each tooth are totalled to provide a score for the entire tooth row, which is then compared to those detailed with respect to age for modern stock. Brown & Chapman (1990; 1991b) devised scoring schemes to assess age from wear in fallow deer and red deer: they examined the permanent mandibular premolars and molars of fifty-three fallow deer ranging in age from four to ninety-one months, and of one hundred and eleven red deer ranging in age from five to one hundred and thirty-eight months (Brown & Chapman, 1991b). Deciduous teeth were not considered because they are all lost within the first 2.5 years of life in both species, and permanent incisors and canines were not considered because they showed few qualitative changes associated with wear. For both species, three main stages of molar wear were observed as follows:

1. Exposure of Primary Dentine - Shortly after each molar comes into contact with the tooth in the opposite jaw, the enamel on its cusps is lost rapidly and the underlying dentine is exposed; mesial cusps before distal cusps, and the mesial slopes of each cusp before the distal slopes. In the third molar the additional third cusp, the hypoconulid, is the last to come into wear and expose dentine.

2. Exposure of Secondary Dentine - With additional progressive wear, a spot or 'eye' of secondary dentine is exposed in the worn dentine surface.
3. Loss of Infundibulum - Eventually, the enamel surrounding the infundibulum of each molar is worn away, and the occlusal surface of each was seen to consist entirely of dentine surrounded by a thin ridge of peripheral enamel.

Similar wearing of the outer enamel and exposure of the underlying dentine was noted in premolars, "but not in the same distinct sequential pattern as for the molars" (Brown & Chapman, 1990, p. 666).

Individual molars were then scored as follows:

- Each slope of a tooth cusp that had just the enamel worn - 1 point.
- Each slope of a tooth cusp that had exposure of underlying dentine - 2 points.
- Dentine between the mesial and distal slopes showed a central white eye - 1 point.
- Dentine exposed to link the dentine of the lingual and buccal cusps - 1 point.
- Mesial and distal cusps so worn that there was a continuous joining line of exposed dentine between the two cusps on either the lingual or buccal aspects of the tooth - 1 point.
- The exposed dentine linking the lingual and buccal cusps, and that linking the mesial and distal cusps was stained dark brown - 1 point.

For the third cusp of the third molar, the 'hypoconulid', scores were given as follows:

- '1 point each for wear on the buccal and lingual sides, and an extra point if the two sides were joined together.'
- 'An additional point if the lingual or buccal wear was continuous with that of the second cusp.'

Finally, for molars that had all the enamel lining the infundibulum worn away 'so that there was a continuous core of dentine surrounded by peripheral enamel' an additional point was given.

Premolars were scored as follows:

- For each ridge that was worn - 1 point.
- When all the ridges of a tooth were joined - 1 point.

Table 1.15: Mean Ages (in months) by Which Specific Permanent Molar Wear Stages Were Noted in Fallow Deer (after Brown & Chapman 1990)

Wear Stage	3rd Molar	2nd Molar	1st Molar
Mesial cusps begin to wear	26-35	>11	4-11
Distal cusps begin to wear	26-35	18-22	4-11
Mesial marginal ridge wear begins	48-59	>22	4-11
Mesial marginal ridge wear always present	>91	26-35	18-22
Hypoconulid wear begins	24	not applicable	
Hypoconulid wear always present	48	not applicable	

Table 1.16: Ages (in months) by Which Specific Permanent Molar Wear Stages Were Noted in Red Deer (after Brown & Chapman 1991b)

Wear Stage	3rd Molar	2nd Molar	1st Molar
Mesial cusps begin to wear	<26	<13	<5
Mesial cusp wear always present	<26	<13	<5
Distal cusps begin to wear	26-33	13-19	5-11
Distal cusp wear always present	38-42	26-33	13-19
Mesial marginal ridge wear begins	26-33	26-33	13-19
Mesial marginal ridge wear always present	87-138	87-138	50-55
Hypoconulid wear begins	26-33	not applicable	
Hypoconulid wear always present	63-66	not applicable	

- When all the underlying dentine was exposed - 1 point.

The total wear score for each mandible was calculated, and a growth curve model was fitted to the total mandibular wear scores that allowed age to be predicted for an unknown jaw specimen. Wear patterns were similar for both fallow and red deer, but the ages at which particular stages of wear were reached were different for the two species (Tables 1.15 and 1.16).

To use the Brown & Chapman scoring scheme to determine the ages of fallow or red deer:

- If all the permanent mandibular molars are available - then calculate the total molar wear score, refer to the relevant logistic growth curve and read off the predicted age together with the 95% inverse prediction limits for that score.
- If only a single premolar or molar is available - then score the tooth and match the score against those detailed (for the appropriate tooth) at differing ages.

At the time of writing Richard Carter (1996), an undergraduate student at the Institute of Archaeology, was developing a scoring system for wear in roe deer based on the standards of Brown & Chapman (1990; 1991b). He kindly supplied wear scores for the roe deer that formed the focus of the current study.

- **Measuring**

The cheek teeth of all cervid species are wider towards their bases than they are towards the crown, thus as wear proceeds these teeth not only become lower but also become broader. The changing dimensions of cheek teeth, caused by wear, has been used in the age estimation of:

- Red Deer (*Cervus elaphus*)
- White-Tailed Deer (*Odocoileus virginianus*)
- Moose (*Alces alces*)
- Rocky Mountain Mule Deer (*Odocoileus hemionus*)

Red Deer (*Cervus elaphus*)

Eidmann (1932, cited in Lowe, 1967), working on red deer, was probably the first to attempt to link age with measurements of tooth dimensions. He examined the first incisors of fifty-eight animals (aged through a visual assessment of tooth wear) and recorded:

1. Crown height
2. Crown breadth
3. Elevation beyond the gum
4. The upright angle of each in relation to the axis of the gum

All measurements were compared with age, but only the figures recorded for crown height showed a link with age and they indicated a decline in height with advancing age. The crown height noted for a yearling was 16.5mm and for a twelve year old it was 7.2mm. However, in each year age class the variation in crown height amongst individuals was too great for use in estimation of age. Lowe (1967), in a study of thirty-four known age red deer from the island of Rhum in the Inner Hebrides, also noted that, although mandibular first molar crown height decreased with age after four years, the variations in heights recorded within different age classes were too great for the measure to be of value in determining age. However, Klein *et al.* (1981) were able to devise formulae for the prediction of age from crown height in red deer, which could be used to place animals into 'broad but useful age classes'.

White-Tailed Deer (*Odocoileus virginianus*)

Severinghaus (1949) attempted to link age and changes in tooth dimension in white-tailed deer. He recorded the crown height of the lingual crests of the premolars and molars in his specimens, and compared the resulting measurements with age. A reduction in crown height with an associated increase in age was identified in the molars, but not for the third cusp or hypoconulid of the third molar, and not in the premolars. The crown height of the first molar's lingual crests in one yearling measured 9.3mm and those in a ten year old measured 4.2mm. As found by Eidmann (above), in each year age class the variation in crown height amongst individuals was too great for a relationship between crown height and age to be established.

Moose (*Alces alces*)

Passmore *et al.* (1955) devised an index that did allow the age of moose to be determined from two measurements of the distal buccal cusp of the second mandibular molar. The two measurements were:

1. The greatest width across the occlusal surface of the cusp, and as averaged from the measurements of the second molars in both the left and right halves of the mandible.
2. The height of the crown, measured from its base or 'point of union with the roots' to the occlusal surface, and again as averaged in both halves of the mandible.

The first measurement was divided by the second to obtain an index value. As crown height shows a reduction with advancing age and crown breadth increases with advancing age, then the index value should increase with advancing age.

Rocky Mountain Mule Deer (*Odocoileus hemionus*)

Robinette *et al.* (1957) also devised a method for age determination, in Rocky Mountain mule deer , based on a ratio of premolar and molar height to width. For each mandible the sum of the widths of all the premolars and molars in one side of the jaw was divided by the sum of their heights, and resulting ratios were found to increase with age (Table 1.17).

Table 1.17: Rocky Mountain Mule Deer Mandibular Cheek Teeth Height to Width Ratios in Animals of Different Age Class (after Robinette *et al.*, 1957)

Age in Years	Ratio	Age in Years	Ratio
2.5	0.33	10.5	0.73
3.5	0.40	11.5	0.80
4.5	0.45	12.5	0.88
5.5	0.50	13.5	0.97
6.5	0.54	14.5	1.10
7.5	0.58	15.5	1.25
8.5	0.62	16.5	1.46
9.5	0.67		

1.1.5.1 The Advantages

- Tooth wear can be recorded rapidly by eye.
- When combined with tooth eruption data, assessments of wear can be used to age animals over the entire age range, from birth to death.
- Jaws may be seriated (that is laid out in order of increasing wear stage) so that a general assessment of a population structure may be made without actually assigning specific ages.
- Deer, like most ungulates, have broad high crowned teeth which pass through many definable and measurable wear stages, and so animals may be placed into quite narrow age classes.
- Scoring defined wear stages and measuring crown height removes the element of subjectivity in recording that is associated with visual assessment of wear.

1.1.5.2 The Disadvantages

- As the structural changes per 1mm of wear in taller less worn teeth are more obvious than those in shorter worn teeth, definable wear stages become less apparent with increasing age, and consequently it is difficult to differentiate between animals in older age classes (Spinage, 1973). For example, in a study of sheep\goat tooth wear Payne (1973) noted that the initial stages of wear each lasted a few months, and so there were many identifiable age classes for the younger animals. However, later stages of wear tended to last for several years each and the older animals could only be placed into broad age estimation categories.
- Visual observations of wear are open to observer errors. Gilbert & Stolt (1970) reported "variation in individual interpretation of wear" in studies conducted by Ryel et al (1961) and Gilbert (1964). Keiss (1969) further compared the age estimates of eleven independent biologists for a series of elk jaws, and found that some estimates were up to 7 years in error, although all were based on the same criteria. Low & Cowan (1963) also found considerable variation between the tooth wear ages assigned to wild mule deer by four different observers (Table 1.19).
- "Animals of the same age class often show wide variations in the extent of tooth wear" (Aitken 1975, 15). Such variations are brought about by differences in diet, nutritional status, heath and

tooth morphology (Chaplin & White, 1969; Hillson, 1986). For example, animals grazing on short dusty vegetation or vegetation grown on sandy soils have a high accidental intake of silica and experience much more rapid wear than those eating comparable vegetation grown on less sandy or dusty soils (Chaplin 1971). Deniz & Payne (1982) attributed the greater and earlier wear shown in two goat herds to the poorer grazing conditions than those of a third flock. Beiger (1939, cited in Anderson 1953) studied tooth wear in roe deer and concluded that wear patterns varied to such an extent between individuals of the same age that the method should not be used to estimate the age for those over 3 years old if an accuracy of better than ± 1 year is required. Similarly, in his study of roe deer Aitken (1975) noted that after 4 and 5 years there was great variation in individual levels of tooth wear, and he stated that the wear patterns he described for the older age class animals "apply to a majority but not all the jaws of a particular age" (Aitken 1975, 24). Tooth wear rates reported by Chapman & Chapman (1970) for British fallow deer are different to those reported by Ueckermann & Hansen (1968) for German fallow deer, and both are also different to those reported by Rieck (1973) for German fallow deer.

- This method of age determination relies upon the survival and recovery of the bulk of the tooth row or 'key' teeth for which much data is known.

1.1.5.3 In Summary

- Shortly after the teeth erupt they begin to wear.
- Wear results in a reduction of tooth crown height, and the removal of enamel and exposure of greater amounts of enamel on the occlusal surface.
- Wear can be assessed by observing the changes manifested in the occlusal surface, by scoring those changes or by measuring reductions in crown height.
- When combined with knowledge of tooth eruption and succession, tooth wear can be used to age animals over their entire age range.

1.2 Incremental Count Methods

Secondary dentine is a continually accruing mineralised tissue that is deposited within the central pulp cavity of teeth, throughout life. Cement is also a continually accruing mineralised tissue, but it is deposited on the tooth roots and, in most high crowned ungulates such as cervids, on the tooth crown. Both tissues have a layered structure, and each layer (or 'lamina' or 'zone' or 'line') is made up of two bands, one broad and one narrow, and the specific appearance of which varies in relation to the observational technique adopted:

- When sections are viewed with transmitted light the broad band is opaque and the narrow band translucent.
- When viewed with reflected light the broad band is dark and the narrow band light (the layers are correspondingly less and more light-scattering).
- When viewed in the scanning electron microscope the broad band forms a ridge and the narrow band appears as a trough.
- When decalcified sections are stained with haematoxylin the broad band stains more intensely than the narrow band.

(Hillson 1986).

Layering has been attributed to seasonal growth changes in the deposition rate of each tissue (Klevezal & Kleinenberg, 1969). In animals of the northern hemisphere the broader band is formed in the summer, between March and November, and the narrow band is formed in the winter, between December and February (Hillson, 1986). Thus usually (but see Laws, 1952 below) each adjacent pair of broad and narrow bands represents one years growth, and the number of pairs of bands in a tissue sample represents its age in years. To age an animal a tooth is sectioned (so that the layers can be examined microscopically) and the number of layers counted. If the tooth used is present at birth then the number of layers should represent the animals age, but if it erupts some time later then an adjusted count must be made. For example, if the tooth examined erupts at 3 years (and the first layer is laid down then), age, in years, is equal to a count of the layers plus 2. The counting of growth layers in secondary dentine and cement is a widely recognised ageing technique and is commonly practiced by wildlife biologists today (Ratcliffe & Mayle, 1992). However, those using counts of incremental layers to estimate age have experienced varying levels of success.

1.2.1 SECONDARY DENTINE

Eidman (1932, cited in Lowe, 1967), working on material collected in Germany, noted that secondary dentine started to fill the pulp cavity in the incisors of red deer after the age of 3 years and, although its deposition was seen to be irregular, its consistency appeared to change with summer and winter seasons to produce a layered structure. It was suggested that the counting of the layers could be used as a method of age determination up to 12 years, after this time the initial layers laid down were gradually lost along with the crown during attrition. Some time later, Lowe (1967) noted the structure of secondary dentine in longitudinal sections of first incisors from known age red deer (between 1 and 7 years old) from the island of Rhum, off the west coast of Scotland. Secondary dentine was present in the tooth of a 2 year old and in the 5, 6 and 7 year olds, but it was not seen in the yearling or in the 3 and 4 year olds. Where it was present, Lowe reported that he could not "discern any obvious differentiation in the dentine 'plug' ascribable to season" and that it did not show "any quantitative correlation with age" (Lowe, 1967, p. 143). However in a footnote to his work, Lowe further reported that a 9 year old stag did have secondary dentine within the pulp cavity of its first incisors which showed clear stratification, but eight layers were observed and not six as expected (Eidmann, above, stated deposition began at 3 years). Thus in this case, secondary dentine showed 'discontinuity in its consistency', and the number of layers was not a reliable indicator of age. Klevezal & Kleinenberg (1969) also reported that annual secondary dentine layers had been noted in the teeth of roe deer (*Capreolus capreolus*) and axis deer (*Cervus nippon*).

1.2.1.1 The Advantages

1) Providing there is sufficient space within the pulp cavity, secondary dentine is a continually accruing tissue and does not undergo resorption. Thus, once layers are deposited they remain throughout the life of the animal and serve as a permanent indicator of its past growth.

2) Counts of annual layers allow the specific age of an individual animal to be calculated, unlike ages determined through comparative methods which only allow the age of an animal to be expressed in relation to the development of another 'control' animal.

3) This method of age determination is especially useful for older animals where other age determination methods often fail (above).

4) Usually protected within an enamel and cement cap, secondary dentine survives in many archaeological contexts, but its structure may be affected (Bell *et al.*, 1991).

1.2.1.2 The Disadvantages

1) In species that do not have continually growing teeth, such as deer, the pulp cavity becomes filled with secondary dentine long before death, and the later deposited layers become extremely compressed and are difficult to isolate and count; 'in such animals counts of secondary dentine layers can lead to serious under-determinations of age' (Stallibrass 1982, 111).

2) Interpretation of a dentine layer sequence many be difficult. Fine accessory bands brought about by short term alterations in growth and tissue deposition caused by pregnancy, lactation and rutting have been noted in secondary dentine. Such layers can be easily and incorrectly recorded as seasonal bands and can lead to over-estimations of age.

3) The technique is destructive - Secondary dentine layers are best observed in cross sections of teeth viewed through a microscope or at least with the aid of a hand lens, thus access to sectioning and microscopic equipment are essential to practice this technique of age determination.

4) The method is most suited to animals over 2 or 3 years old, as in younger animals the permanent teeth may not have erupted, and Klevezal & Kleinenberg (1969) further reported that the layers of the first two years of growth can often be poorly defined and difficult to distinguish.

5) Primary dentine also has a layered structure and consists of both von Ebner's lines and wider lines called Contours of Owen. To the inexperienced such lines could be mistaken for annual layers. Similarly, interglobular dentine also has a layered but not annual layered structure and its presence may add further confusion.

1.2.2 CEMENT

Sergeant & Pimlott (1959) were the first to note the presence of annual layers in the dental cement of terrestrial mammals, and they reported thin translucent zones of cement alternating with thick opaque zones in intact polished sections taken (viewed in transmitted light) from the first incisors of moose (*Alces alces*). The opaque zones had more cementocyte lacunae, which were attributed to the faster growth of these zones. The number of zones were found to correspond with the age (as aged by dental eruption and wear) of individuals from which the teeth came, and for the younger specimens cement zone counts allowed specimens to be aged to within ± 1 year and for the older animals to within ± 2 years.

Low & Cowan (1963) investigated the potential of age determination based on counts of cement layers in the first incisors of wild mule deer (*Odocoileus hemionus*), and then applied their developed technique to 20 known age Columbian black-tailed deer (*Odocoileus hemionus columbianus*) and to a series of teeth from 17 wild mule deer, aged through tooth wear. The first incisor was used as it has a single root and, so, was described as easier to handle than double-rooted molars and premolars and, as it is also the first permanent tooth erupt (at around 12 months), the number of its cement layers is most likely to approximate actual age than those of any other tooth. Various methods for section preparation and microscopic examination were tried, but the greatest detail was seen when decalcified, 10μm thick sagittal sections were stained with Erlich's haematoxylin and examined with polarised light. Annual layers were observed and each was found to consist of 'a pale staining zone of rapid increment', formed during the spring and summer, and a narrower 'dark staining zone' formed during winter. As the first incisor erupts at 12 months and all fawns were presumed born in June, the first zone is laid down during the animals second summer and is followed by a narrow winter zone during the animals second winter, and so on - thus age equals a count of each pair of summer and winter zones +1. By observing the nature of the outer zone the technique could be used to estimate age to within 6 months, and if month of death was known (common in wildlife management, but not in archaeology) then the technique could be used to estimate age to within 1 month. Some of the teeth from the wild deer contained two zones of light staining and two zones of

Table 1.18: Comparison Of True Ages And Cement Layer Ages Estimated For 20 Columbian Black Tailed Deer, By Low & Cowan (1963) (ages are in months)

Specimen	True Age	Cement Layer Age
4354	15.0	17
4348	15.0	16
4347	15.0	16
3528	15.0	16
4265	15.0	18
6713	17.5	19
4189	20.5	21
M-3	24.7	23
6539	29.0	29
4231	34.0	34
L-96	35.9	36
56	35.9	36
L-61	36.1	35
L-55	36.2	36
L-53	36.3	35
4538	40.0	40
8003	41.2	40
8005	53.0	52
6715	60.0	60
4263	72.0	72

Table 1.19: Comparison Of Ages Estimated Through Tooth Wear, By Four Observers, and Cement Layer Ages, By Low & Cowan (1963), In Wild Mule Deer (ages are in months)

	Wear Age Estimates Of Four Observers				
Specimen	**1**	**2**	**3**	**4**	**Cement Layer Age**
14	18	27	18	18	30
15	18	18	18	18	18
16	18	18	18	18	18
17	30	30	30	30	30
18	42	30	42	42	42
19	42-54	30	42	42	42
20	54-68	52	66	42	66
21	78+	102	78	42+	90
22	90+	66	66	72	114
23	66	54	54	54	66
24	114+	138	126	144	174
25	42	42	42	42	42
26	78-80	90-102	66	42	66
27	90+	138+	120	120	132
28	102	102+	102	132	90
29	66	90	66	102	78
30	150+	126+	144	-	162

dark staining cement for each years growth. However, the second pale zone was very narrow and just split the winter dark zone into two. It was reported that the difference between this condition and the normal one was obvious and that it did not interfere with age estimates. Good agreement between cement layer age and known age was found for the Columbian black-tailed deer and most animals were aged to within 1 month (Table 1.18), but much greater variation was shown between tooth wear age (as estimated by four different observers) and cement layer age for the wild mule deer (Table 1.19).

Mitchell (1967) used cement layering to age red deer (*Cervus elaphus*). For 22 animals, aged between 1½ and 19½ years (only two were > 3½ years), a count of one layer (consisting of 1 opaque band and 1 translucent band) allowed age to be assessed to within ± year for 18 animals, but for the two animals aged over 3½ years the technique did not work.

Lowe (1967) tested the cement layer age technique on the mandibular first molars of 34 known age red deer, from the island of Rhum, off the west coast of Scotland. Only half of the animals could be accurately aged using the technique, and the others were either under or over aged due to a deficit or excess of cement layers. For 28 animals aged over 1 year cement layer age estimates were compared with age estimates based upon observations of dental eruption and wear: The cement layer estimates were only correct to within ± 7 months for between 46% and 54% of all cases, but the dental eruption and wear estimates were correct to within ± 7 months for 89% of all cases. Similarly, Gasaway *et al.* (1978) found that, for 45 moose all of known age >2 years, less than 56% of cement ages were correct.

Aitken (1975) examined cement layers in the first mandibular molars of 47 roe deer of unknown age from Thetford Chase, Norfolk. Classic 'alternating broad white summer-deposited and narrower dark winter-deposited bands' were noted in the cement layers. The broad white bands consisted of cementocytes and the narrower dark bands of amorphous cellular material. Aitken stated that the cementocyte bands were of 'even thickness and deposited in a regular fashion' and, thus "gave a strong indication that they were laid down at the rate of one a year and could ... be used as a guide to the age of animals" (Aitken, 1975, p. 19) (below). Several does showed 'broad white summer bands' which were interrupted by very narrow dark bands, and these breaks in cementocyte deposition were attributed to physiological stresses, such as lactation, which occurs between May and September. Some bucks also showed interrupted cementocyte band deposition, and this was attributed to the August rut. The cement in an additional five known age specimens was further examined:

1. 2 year old from Dorset

   Cement was present but it contained a mass of cementocytes and no clear band formation.

2. 4 year old from Dorset

   Cement contained four thick white cementocyte bands separated by dark acellular bands. The three outermost bands 'were interrupted by a thin intermediate line of acellular cement'. As this animal was a buck, the interruptions were seen to represent 'rutting bands'.

3. 4 year old from Dorset

   Cement contained four white bands of cementocytes separated by dark acellular bands.

4. 7 year old from Dorset

   Cement contained seven white bands of cementocytes, which were poorly defined due to the 'scant development of the intervening acellular bands'. The white bands were not continuous over the cut face of the cement and in some areas only 6 bands could be seen.

5. 3 year old from Surrey

   Cement contained two thick white bands of cementocytes and the beginnings of a third. As above, the white bands were poorly defined due to the scant development of intervening acellular bands.

Generally, the five known age animals had poorly defined cementocyte layers but "whenever a consistent number of bands could be seen it always corresponded to the actual age of the animal" (Aitken, 1975, 20), and this led Aitken to conclude that the layers seen in the Norfolk deer were annual and could be used to estimate age.

The cement layer method used to the age the roe deer which form the main focus of this study (Chapter 4) was described by Ratcliffe & Mayle (1992), and is summarised below:

1. If necessary clean excess soft tissue from the mandible.
2. Clamp the mandible in a vice and use a fine hacksaw to cut the second molar in half, to a depth of at least 0.5cm into the mandible.
3. Remove the front portion of the tooth and polish the cut surface with fine emery paper. Wipe the polished surface with a moist cloth to remove any loose abraded particles.
4. Support the tooth, polished side up, in Plasticine, Blu-tak or similar, on a small piece of wood or plastic that can be moved around on a microscope stage. Illuminate the polished surface and examine at a magnification of between x10 and x40.
5. Locate the cement pad, at the fork in the tooth roots, and count the broad white bands. Each band represents one summer's growth. The first band is laid down at around 1 year old, during the animals second summer.
6. If the bands are indistinct, across any part of the cement pad, repolish the tooth and/or move it around under the microscope and/or alter the angle of illumination until the maximum number of layers can be counted. If the clarity is still poor the posterior part of the tooth or the second molar from the other half of the mandible should be examined. The counted number should equal the animal's age, in years.

It was further explained that if the month of death is known then age can be estimated to the nearest month (assuming a common birth date of 1st June). For example a doe shot on 1st December with four white broad bands will be 4 years and 6 months old. Finally, it was noted that the technique has several inherent problems:

- Adult bucks showed fine discontinuous layers within the broad white layers, also noted by Lowe & Cowan and Aitken (above), and, again, were attributed to disruptions in growth brought about by the annual summer rut.
- For animals killed during the summer, confusion can arise in assessing their age, as the current years broad white band may not have begun to form.

1.2.2.1 The Advantages

1) Like secondary dentine, cement is a continually accruing tissue and does not undergo resorption, thus again once layers are deposited they remain throughout an individual's life.
2) Again, counting of cement layers allows the absolute age of an individual animal to be assessed.
3) Cement layer counting is particularly suited to deer and ungulates in general. Deer teeth have a maximum crown height, but to compensate for the effects of wear and to maintain occlusion their teeth are pushed high above the gum line through the continual eruption of the roots. Most external cement is deposited on that part of the tooth closest to the gum line to ensure that the tooth is securely anchored within the mouth. Continual eruption results in ever thickening annual cement deposits around the base of the tooth, these layers are not spatially limited as secondary dentine layers are and are therefore easily studied.
4) In theory, this method of age estimation is particularly suited to older animals where other methods of age determination often fail (above), however it has not been tested on animals over 7 years (Aitken, 1975).

1.2.2.2 The Disadvantages

1) Cement, like secondary dentine can show fine accessory bands which are not related to age and can lead to over-estimations of age.
2) Again, in younger animals the best teeth to use may not have erupted, although in roe deer all teeth have erupted by the end of the first year.
3) To observe cement layers in teeth requires sectioning, and so access to laboratory equipment and relevant microscopic instruments are needed.

1.2.3 In Summary

- Secondary dentine and cement has a layered structure, and the number of layers present often reflects age.
- No single causal factor has been attributed to the development of such layers (Hillson 1986), although there is a general agreement that variations in seasonal growth do contribute to their

periodicity and clarity (Klevezal & Kleinenberg, 1969; Stallibrass, 1982). However, other causal factors, such as diet, pregnancy and the rut, have been linked to the development of accessory layers in dentine and cement, and the presence of such accessory layers can lead to 'false age counts'.

1.3 In Conclusion

The traditional age estimation techniques, discussed above, have limited application to cervids, especially roe deer - the species which forms the main focus of the current project. Some techniques are restricted to the study of a particular age group of animals (such as juveniles or adults), others are limited to the study of well-preserved unfragmented skeletal remains and some are limited by both restrictions:

- Currently, fusion and bone growth can only be used to age roe deer up to 30 months old, and the method requires the recovery of the epiphyseal regions of the phalanges and/or metapodials (Table 1.3).
- Antler growth cannot be used to determine the specific age of roe deer, but it may be used to make a relative assessment of maturity. Only bucks carry antlers, and the method requires the recovery of intact, unshed antlers; that is those still attached to the pedicle.
- Age-related tooth development data are not yet available for roe deer and, as roe deer permanent tooth eruption is completed towards the end of the 1st year of life, the value of such data would be extremely limited.
- Tooth eruption and succession may only be used to estimate the age of roe deer up to 1 year old, after this time the full permanent dentition is complete. For the most precise age estimates, the method requires the recovery of jaws with complete cheek tooth rows intact.
- Patterns of tooth wear have been described in roe deer up to 8 years old (Aitken, 1975), and a scoring method is currently being developed that, it is hoped, will be applicable to specimens of all ages (Carter, 1996). However whatever the recording method, the most precise estimates of age are made when jaws are recovered with complete cheek tooth rows intact.

- Theoretically, counts of incremental layers in secondary dentine and cement can be used to estimate the age of roe deer from the eruption of their permanent teeth (1st year of life) and throughout their natural life span. However, although layered secondary dentine has been observed in the teeth of roe deer (Klevezal & Kleinenberg, 1969), its accuracy as an age indicator has not been assessed. Similarly, there appears to be only one study that tested the accuracy of ages based on counts of cement layers in roe deer (Aitken, 1975). In this study four out of five animals had a count of 'broad, white cementocyte bands' that corresponded to their known age (in years), but the clarity of the layers was poor and counts were hindered by the presence of rutting lines (interruptions within the broad white bands). Finally, counts of secondary dentine and cement layers are dependent upon the recovery of intact teeth, unaffected by diagentic changes (Chapter 4).

Consequently, there is a need for either the refinement of the two existing age estimation techniques that have the potential to be applied to roe deer of all ages (tooth wear and incremental count techniques), or for the development of a new technique that is not limited in its application to the study of teeth. Here a new technique is proposed; that is the quantification of histological features within cortical bone - a technique which has long been used by forensic scientists and anthropologists to age humans (Chapter 3). In the next chapter the development and structure of those histological features within bone that can be quantified and used to estimate age in humans is discussed.

CHAPTER 2 BONE HISTOLOGY

Archaeologists often forget that excavated bones were once highly vascularised living connective tissues, existing in a constant state of regeneration or turnover. In general, connective tissues are fibrous tissues that support, bind or separate other tissues. In the mammalian skeleton bones serve to support the body, protect the soft tissues, such as the brain and the bone marrow, and provide a source of mineral ions and calcium, that can be diffused into the blood as necessary. This chapter is concerned with a discussion of bone biology and micro-anatomy, *in vivo,* essential to the understanding of the formation of those histological features which may be quantified and used to estimate age - the ageing technique which forms the focus of the project. Topics included are:

2.1 Bone Microstructure

2.2 Bone Types

2.3 Bone Development

2.4 Bone Turnover (Remodelling)

Unless specified, spellings of anatomical and biological terms follow those used by Gray (1995).

2.1 Bone Microstructure

Bone consists of:

2.1.1 Cells

2.1.2 Blood Vessels, Nerves And Surface Tissues

2.1.3 Acellular Matrix

2.1.1 CELLS

The three main cell types active within bone are:

2.1.1.1 Osteoblasts

2.1.1.2 Osteocytes

2.1.1.3 Osteoclasts

2.1.1.1 Osteoblasts

Osteoblasts are bone forming cells, responsible for the production and mineralisation of the proteinaceous acellular matrix of bone (below). They are approximately cuboid cells, varying in size between 15μm and 30μm across and containing between one and three oval nuclei. The nuclei are supported by a well developed granular endoplasmic reticulum and a large Golgi apparatus, both of which are features of protein secreting cells. Osteoblasts also, characteristically, contain substantial amounts of the enzyme alkaline phosphatase. The specific role of this enzyme in bone formation, if any, is not fully understood but its activity is correlated with bone formation (Puzas, 1993). The underside of each osteoblast sits on the existing bone surface and from it arise numerous finger-like cytoplasmic processes, through which the intracellularly produced bone proteins are exported. Osteoblasts do not occur in isolation but are found clustered together in working layers - one cell deep but consisting of expanses of up to four hundred cells. The osteoblasts secrete fresh matrix, through their cytoplasmic processes, between themselves and the previously existing connective tissue surface. They are highly mobile cells, and they are able to move away from an area after completing the required matrix secretion. Fresh bone matrix is not mineralised and is referred to as osteoid. Mineralisation of osteoid does not commence immediately after its deposition, and there is a time-lag or maturation period before it occurs. In humans the time-lag is between five and ten days. Osteoblasts do play a role in mineralisation, by secreting mineral ions and fixing them into the proteinaceous framework of bone (below).

Osteoblasts originate from preosteoblasts. These are elongated cells that contain a single lengthened nucleus, but which lack the well developed granular endoplasmic reticulum and large Golgi complex of mature osteoblasts. They are found in layers close to active osteoblasts, and proliferate through division. Mature osteoblasts are not capable of division or proliferation. They gradually take on the appearance, and develop the characteristic protein secreting mechanisms of mature osteoblasts, as they move closer towards bone forming surfaces and join the existing layers of active cells. Inactive osteoblasts take on a flattened outline and a proportion are enveloped within mineralising osteoid tissue, to become osteocytes.

2.1.1.2 Osteocytes

Between 10% and 20% of all osteoblasts are enclosed within mineralising osteoid to become osteocytes. The spaces occupied by the cells within the matrix are called lacunae. Osteocyte lacunae are oval shaped and arranged so that their long axis is parallel to the long axis of adjacent collagen fibres in the matrix (below) and their shortest axis is transverse to them. Calcium saturated extracellular fluid flows between each osteocyte and the unmineralised osteoid walls of its lacuna. Osteocytes are found scattered throughout the bones of most vertebrate species, although acellular bone has been noted in some fish species (Enlow & Brown, 1956; 1958), and are connected to each other (and to active osteoblasts) through an irregular network of tiny radiating cytoplasmic processes contained within tunnels called canaliculae. These are remnants of the cellular processes that extended from osteoblasts during bone mineralisation. The precise function of the canaliculae is unknown, but it is generally accepted by histologists (Gray, 1995) that they form a network for nutrient diffusion and permit "communication" between connected cells. Newly formed osteocytes are plump and roughly rounded, and retain many features of active osteoblasts, but they are much less metabolically active than osteoblasts and as they age there is a gradual reduction in their protein synthesizing features (granular endoplasmic reticulum and Golgi complex). Mature osteocytes are oval shaped and measure, approximately, 25μm long. Like osteoblasts, osteocytes are not capable of proliferation or cell division and their function is unclear. It was thought they could resorb bone through enlarging their lacuna, but in recent years such osteocytic osteolysis has been shown to be a fixation artefact (Boyde, 1980) and, in contrast, it has been demonstrated that osteocytes are capable of producing transforming growth factor β (TGF-β), a hormone which can stimulate bone formation. Whatever their precise function, it should be noted that death of osteocytes leads to localised bone resorption, so they are vital for maintenance of the tissue.

2.1.1.3 Osteoclasts

Osteoclasts are responsible for bone removal or resorption, a key process in remodelling (below). They are large rounded cells approximately 20μm in diameter, and each one contains between fifteen

Table 2.1: Mean Femoral Haversian Canal Perimeter in Man and Some Other Animals (after Jowsey, 1966).

Specimen	Mean Femoral Haversian Canal Perimeter (μm)
Rat	36 ±12
Cat	102 ±36
Dog	85 ±37
Monkey (Rhesus)	167 ±46
Man (adult)	173 ±45
Cow (adult)	213 ±47

This table should not be used as a guide for species identification as other factors, such as the angle of section, can effect the appearance of Haversian canal dimensions, and because of the considerable scope of variation identified.

and twenty oval nuclei. Osteoclasts work in isolation, and are only seen on surfaces where they are actively removing bone. The underside of each osteoclast, which is in contact with the bone surface, has a ruffled appearance and is made up of irregular cell extensions called lobopodia. Osteoclasts resorb bone by releasing intracellularly produced proteolytic enzymes and hydrogen ions through the lobopodia. These substances react with lysosomal enzymes, which are also released by the osteoclasts to form the perfect environment for bone degradation. Initially, the secreted enzyme and ion solution causes demineralisation of the bone, then it dissolves its proteinous framework. The resorptive area around the lobopodia is delimited by a peripheral zone of actin filaments, which also serve to attach the osteoclast to the bone surface. Like osteoblasts, osteoclasts are highly mobile cells, and they advance across bone surfaces leaving a trail of scalloped hollows (resorption hollows), or Howship's lacunae, behind them. Osteoclasts arise from the fusion of single-nucleus precusor cells present in bone marrow and, as they are never seen in a resting state, it is presumed that after completing resorptive activity they sub-divide and revert back to the haemopoietic mononuclear cells from which they originated.

2.1.2 Blood Vessels, Nerves And Surface Tissues

Bones are permeated by a complex network of blood vessels and nerves. The blood vessels supply bone and its cells with essential nutrients, and the predominant orientation of the major blood vessels varies with bone maturity. They appear randomly orientated within the matrix of juvenile bone and interconnect and radiate in all manner of directions. In adult bone they are predominantly aligned along the bones and are known as Haversian canals, named after Clopton Havers (1692) who first identified them. Haversian canals are surrounded by individual layers of bone called lamellae (below). Haversian canal size (Table 2.1) has been shown to vary with respect to species (Jowsey, 1966). Other canals which run perpendicularly to the long axis of bones can also be found in adults. These are known as Volkman's canals, and they serve to interconnect Haversian canals. Volkman's canals are smaller than Haversian canals and they are not surrounded by concentric lamellae.

Nerves are most abundant in the articular ends of long bones, in the vertebrae and in the larger flat bones. They are also found in the perivascular spaces of Haversian canals, and in the periosteum and endosteum. The periosteum is a fibrous tissue that surrounds bone, but not on articular surfaces, and it is connected to it through coarse bundles of collagen fibres known as Sharpey's fibres. These fibres, along with other loosely arranged connective tissue and osteoprogenitor cells, form the inner layer of the periosteum. When activated, the cells are capable of differentiating into preosteoblasts and preosteoclasts. The outer layer of the periosteum is made up of dense fibrous connective tissue. The endosteum is a fibrous tissue that lines the central marrow cavities of bones. It is thinner than the periosteum, as it only consists of a single layer of condensed connective tissue and osteoprogenitor cells. Again, when activated its cells are capable of proliferation to form preosteoblasts and preosteoclasts.

2.1.3 The Acellular Matrix

The acellular matrix of bone consists of:

2.1.3.1 Collagen Fibres

2.1.3.2 Ground Substance

2.1.3.3 Minerals

2.1.3.1 Collagen Fibres

Collagen is a fibrous protein which is found in all connective tissues. There are fourteen types of collagen, but only one type, Type I, is found within bone. It forms approximately 21% of the dry weight of bone and comprises up to 90% of all bone proteins, and it is the most abundant form of collagen in most other connective tissues. Individual collagen fibres are made up of adjacent chains of collagen molecules packed end to end, but with short spaces or gaps between them which become filled by crystallites of apatite (below) during bone mineralisation. The many intra- and intermolecular cross-links of collagen render it highly insoluble, and serve to provide bone with a tensile strength that enables it to withstand stresses and strains. In acidic burial conditions collagen survives quite readily, and it has even been identified in bones that have undergone the complex chemical process of fossilisation (Brothwell, 1981). However, in alkaline to neutral conditions collagen decays rapidly and only the mineral content of bone survives. Similarly, if the molecular structure of bone collagen is altered, as it is when heated during cooking, bones become brittle and fracture easily as their mechanical strength and resilience is lost. In archaeological bones, the protein usually accounts for less than 10% of the total dry weight, and if they account for 5% or more preservation is good.

2.1.3.2 Ground Substance

Ground substance is the name given to the organic matter that lies between collagen fibres. Histologically, it can be made visible through staining (Hancox, 1972) and, chemically, it has been found to consist of non-collagenous proteins (NCPs), small amounts of lipids (about 0.1 wt% of the whole tissue) such as triglycerides, and other organic molecules including citrate and lactate (Williams & Elliott, 1989). Most NCPs, such as proteoglycans, are complex acidic (anionic) polymers which contain protein and carbohydrate. NCPs adhere to collagen fibres and provide attachment locations for bone cells, and they are also thought to be involved in the mineralisation process. Like collagen, most of the NCPs found within the accellular matrix of bone are produced by osteoblasts, but approximately 25% of them are not and are either absorbed or trapped within osteoid during its mineralisation. For example, α_2-HS-glycoprotein is a plasma rich NCP which is synthesized in the liver but which is absorbed into bone matrix during mineralisation.

2.1.3.3 Minerals

The mineral (inorganic) element of bone is in the form of crystallites of apatitic calcium phosphate. These crystallites, which are initiated by osteoblasts, bind with the organic framework (collagen fibres) of bone and provide it with its rigidity. The calcium phosphate of bone is very closely related to hydroxyapatite, but the inorganic fraction of bone has a more variable calcium/phosphorus ratio than hydroxyapatite, and also contains water and several ions such as sodium and fluorine which are absent in hydroxyapatite. Apatite crystallites are insoluble at normal body pH, but in dilute acids or calcium chelators such as ethylene diamine tetracetic acid (EDTA) they can be dissolved.

2.2 Bone Types

Two main types of bone are recognised on the basis of their microscopic structure:

2.2.1 Woven Bone

2.2.2 Lamellar Bone

When viewed macroscopically these two bone types may appear to form either:

2.2.3 Cancellous Tissue

2.2.4 Compact Tissue

2.2.1 WOVEN BONE

Woven bone is the fastest-growing bone type and it is characterised by an apparent lack of structural order. When viewed under the microscope, it can be seen that:

- Its collagen fibres are coarse and they, along with its apatite crystallites, have no clear orientation or alignment.
- Its osteocyte lacunae are randomly scattered.
- Its blood vessels, called 'non-Haversian canals', have no ordered orientation and appear to interconnect randomly.
- It has comparatively large vascular spaces.

Most mammalian bones are initially laid down in embryo as woven bone. However, at some time during life it is usually resorbed by osteoclasts and is replaced with lamellar bone (below). Some woven bone does persist throughout life in the sutures of cranial flat bones, in tooth sockets and on surfaces where tendons are attached. Woven bone is also the first type of bone to be deposited in post-foetal osteogenic situations, such as in the healing of fractures.

2.2.2 LAMELLAR BONE

The greater proportion of most adult mammalian skeletons consists of lamellar bone. In contrast to woven bone, it appears highly structured when it is viewed microscopically:

Plate 2.1: The Arrangement Of Bone Lamellae

The plate shows an SEM image of a cross section of a roe deer mandible,

in which the three types of bone lamellae can be seen.

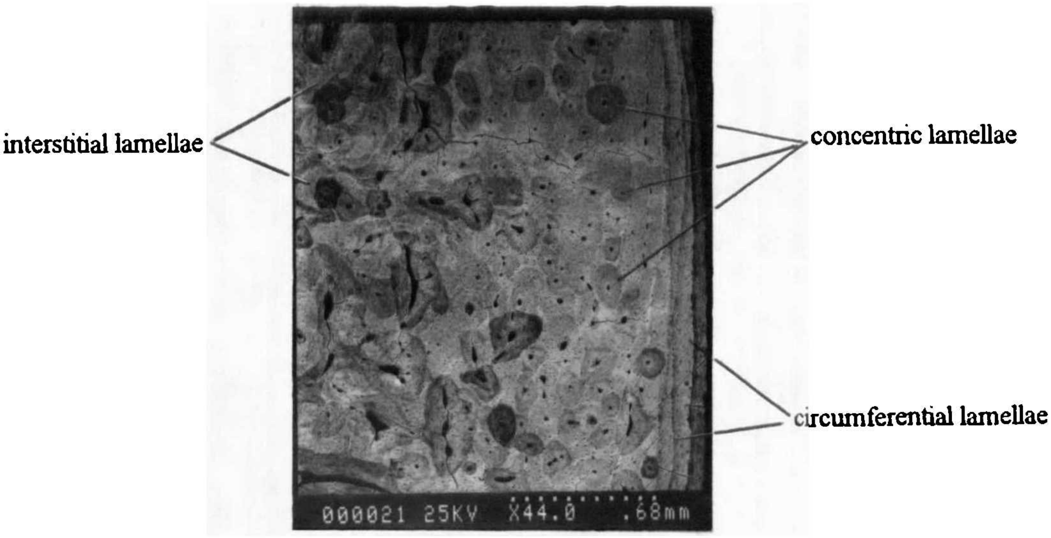
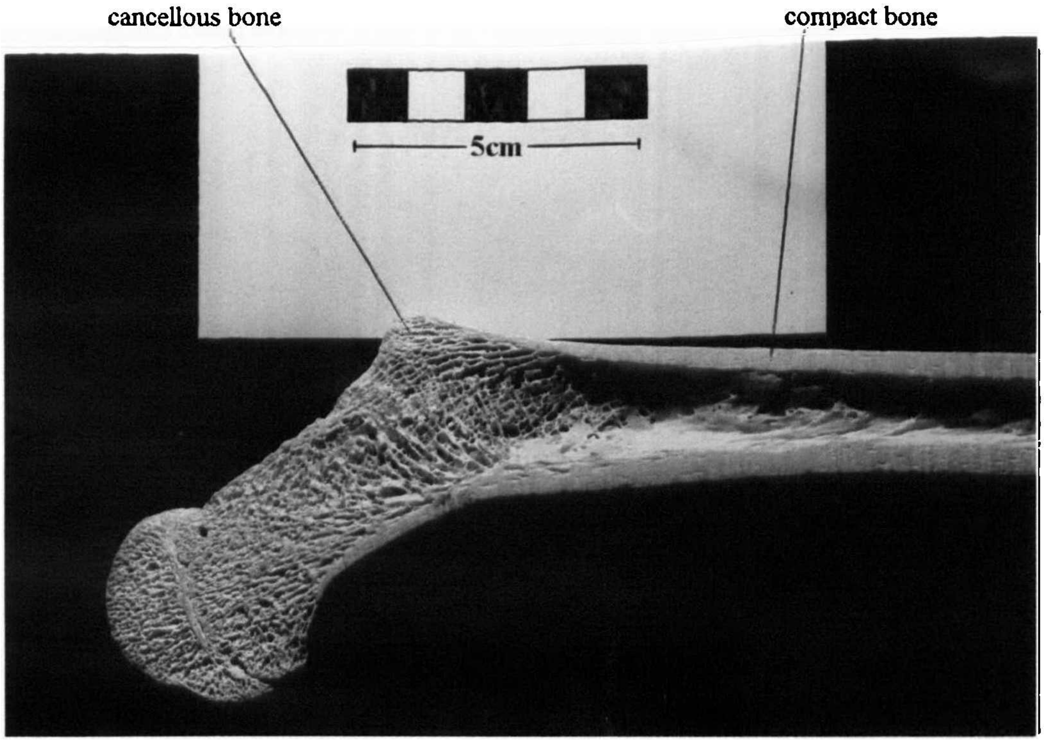


Plate 2.2: Distribution Of Cancellous And Compact Bone

The plate shows a longitudinal section of the proximal end of a human femur,

in which outer compact bone and inner cancellous bone can be seen.



- Its collagen fibres appear fine and, along with its apatite crystallites, are arranged in parallel lamellae, between 3μm and 7μm thick (Junqueira *et al.*, 1995).
- Its osteocyte lacunae are organised into layers, in and between the lamellae.
- Its major blood vessels are housed within Haversian canals and these run parallel to the long axis of the bone.

Lamellae stand out from each other due to the orientation of their collagen fibres for, although the fibres within a single lamella are parallel to each other, those in adjacent lamellae align in a different direction. However, studies with the scanning electron microscope have shown that groups of collagen fibres are not confined to a single lamella, but that they twist and separate into adjacent lamellae (Boyde & Hobdell, 1969). There are three main arrangements and locations within bones in which lamellae are found (Plate 2.1):

1) Circumferentially, around periosteal and/or endosteal bone margins.
2) Concentrically, around Haversian canals. This arrangement of a Haversian canal surrounded by concentric lamellae is called an osteon, or 'Haversian system' (Chapter 2.4.1.2). Some osteons are delimited around their outermost lamella layer by a ring of an amorphous material that consists of dense mineralised matrix with some collagen fibres called a cement boundary or reversal line (this line marks were previous bone resorption stopped and new bone deposition began), these osteons are referred to as secondary osteons. Haversian canals surrounded by concentric lamellae but without a reversal line are known as primary osteons.
3) Interstitially, between osteons.

2.2.3 CANCELLOUS TISSUE

Cancellous bone tissue consists of comparatively large and irregularly shaped vascular spaces, containing fat, blood vessels and connective tissues, which are separated by thin walls of either woven or lamellar bone matrix called trabeculae. In cancellous bone the aggregate volume of the vascular spaces equals or exceeds that of the bone matrix itself (Hancox, 1972), and it is generally found in the interior of bones or within the epiphyseal regions of long bones (Plate 2.2).

2.2.4 COMPACT TISSUE

In compact bone tissues the aggregate volume of actual woven or lamellar bone greatly exceeds that of the sparse and comparatively small vascular spaces. Compact bone is generally found in the outer wall regions of mature bones (Plate 2.2) and provides them with their strength.

2.2.5 IN SUMMARY

- Bone may lack an ordered microscopic arrangement and be known as woven bone, or it may be highly ordered and be known as lamellar bone.
- Macroscopically, both woven and lamellar bone can have few small vascular spaces to form compact bone tissue, or they may have many large vascular spaces to form cancellous tissue.

2.3 Bone Development

Osteoblasts can only secrete osteoid on to exposed surfaces within or on existing connective tissues. When secreting osteoid during continued growth, osteoblasts have an existing bony framework on which to work, but in the absence of such a framework (before any bone has been formed) there are two other connective tissues within the matrix of which they can deposit osteoid:

2.3.1 Mesenchyme

2.3.2 Cartilage

Deposition of bone within mesenchyme is called:

2.3.3 Intramembranous (Mesenchymal) Ossification

Deposition of bone within a cartilaginous matrix is called:

2.3.4 Endochondral (Cartilaginous) Ossification

2.3.1 MESENCHYME

Mesenchyme is an embryonic, jelly-like connective tissue that contains irregularly branching blood vessels and scattered undifferentiated cells. These cells are capable of division, and can differentiate into cells associated with bone such as osteoblasts and osteoclasts, or cells associated with cartilage such as chondroblasts (below).

2.3.2 CARTILAGE

Cartilage consists of:

2.3.2.1 Chondroblasts

2.3.2.2 Chondrocytes

2.3.2.3 Acellular Matrix

2.3.2.1 Chondroblasts

Chondroblasts are the main cartilage producing cells. They are found embedded within cartilage matrix they have secreted (in lacunae) and on surfaces of cartilage masses, where they secrete fresh matrix. They are flat, multi-nuclear cells and they have many fine surface projections called filopodia through which they secrete cartilage matrix. Also, as is characteristic to and essential for protein secretion, they house a well developed granular endoplasmic reticulum and a large Golgi apparatus. Chondroblasts originate from three sources:

1) From precursor cells within condensed mesenchymal tissue (above).
2) From precursor cells within a perichondrium (below).
3) From active (i.e. cartilage secreting) 'parent' chondroblasts, which are capable of proliferation through division.

Several chondroblasts may be noted in a single lacuna within the cartilage matrix, and groups such as these are referred to as cell nests or isogenous groups. Each isogenous group represents the offspring of a single 'parent' progenitor chondroblast. However, multiple cells within a lacuna are soon separated by an ever thickening band of matrix that they secrete themselves, and eventually all chondroblasts occupy their own lacunae in which they become parent cells. As chondroblasts are buried deeper within the matrix they have secreted they gradually enlarge and become more rounded (their lacunae change shape to accommodate them) and loose the ability to divide. Such mature chondroblasts are called chondrocytes.

2.3.2.2 Chondrocytes

Chondrocytes are 'mature' chondroblasts. They are initially capable of matrix secretion, but they do not possess the ability to divide or self-proliferate, and are found buried deep within cartilage matrix, each occupying its own lacuna. They are rounded cells with a single, centrally placed nucleus which is supported by a well developed granular endoplasmic reticulum and a large Golgi complex. However, as chondrocytes become buried deeper and deeper within cartilage matrix their protein secreting features become reduced and they lose the ability to produce and secrete fresh cartilage.

2.3.2.3 Acellular Matrix

The acellular matrix of cartilage consists of Type II collagen fibres, elastin fibres and other minor proteins and lipids embedded in a firm gel rich in carbohydrates. The relative proportions of these acellular features varies between the four different cartilage types: hyaline, elastic, fibro- and articular cartilage. Hyaline cartilage is the type in which osteoblasts secrete osteoid during endochondral ossification. It has a 'glassy, bluish, opalescent homogenous appearance, firm consistency and some elasticity' (Gray, 1995) and its matrix consists of fine fibrils and fibres of Type II collagen between 10µm and 20µm in diameter.

Several other factors regarding cartilage are worthy of note:

1) Its vascular supply is restricted to its surface and to some large penetrating tunnels.
2) It can grow by both interstitial expansion and by surface apposition (below).
3) It is covered by a fibrous sheath called a perichondrium (but not at osseous and synovial surfaces), this consists of highly vascularised layers of collagen fibres and undifferentiated cells, which are capable of proliferation through division and differentiation into chondroblasts, osteoblasts and osteoclasts.

2.3.3 Intramembranous (Mesenchymal) Ossification

Intramembranous ossification involves the direct secretion of osteoid tissue (by osteoblasts) into areas of condensed mesenchymal tissue. It is the process by which the flat bones of the cranial vault and parts of the mandible are formed (below). Each stage of the process is highlighted below:

1) In the foetus, in an area where a flat bone is to form, cells of mesenchymal origin gather around a capillary network, and differentiate into osteoprogenitor cells.
2) These osteoprogenitor cells develop into preosteoblasts and then into osteoblasts.
3) The osteoblasts then secrete osteoid tissue between themselves and around the capillary network.
4) The osteoid tissue 'matures' and fixation of apatite crystallites (mineralisation of the osteoid to form bone) soon follows.
5) Some osteoblasts are trapped within the ensuing matrix and become osteocytes.
6) Surrounding mesenchymal tissue is pushed outwards by the expanding bony mass and it (the mesenchyme) transforms into a protective periosteum.
7) A bone forming in this way continues to grow through surface apposition, through the action of osteoblasts which differentiate from cells deep within the periosteum.

2.3.4 Endochondral (Cartilaginous) Ossification

Endochondral ossification involves the replacement of cartilage templates of bone with actual bone tissue (the cartilage first being laid down by chondroblasts in areas of condensed mesenchyme). It is the process through which much of the mammalian post-cranial skeleton and the mandibular ramus (below) is formed, and it is also the process through which fractured or broken bones are repaired. Endochondral ossification is a complex process and is best understood when considered with respect to long or limb bone development. It should be noted that limb bones consist of three distinct anatomical elements - a main body or shaft which is known as a diaphysis and two ends known as epiphyses. Each stage of limb bone formation (endochondral ossification) is considered below:

1) In an area where a limb bone is to develop, mesenchyme condenses into a rod like structure that approximates the shape of the bone that is to replace it, and the cells within it gather together, commence division and differentiate into chondroblasts.

2) The chondroblasts secrete cartilage matrix around themselves, and within the intercellular mesenchyme.

3) Some chondroblasts become trapped within lacunae in the matrix they have secreted, and they continue to divide and secrete cartilage for some time.

4) The mesenchyme is pushed outwards and transforms into a periochondrium. Centrally located chondroblasts become trapped within lacunae in the cartilage matrix they have secreted, but continue to divide and secrete cartilage for sometime.

5) Cartilage proliferation continues internally through the actions of trapped, continually dividing chondroblasts and externally through the activity of chondroblasts that have differentiated from the surrounding perichondrium.

6) As the cartilage template continues to grow, chondroblasts within the most central, earliest formed areas of cartilage become transformed into chondrocytes and lose their ability to continue matrix secretion.

7) Gradually, the chondrocytes degenerate and die, and the walls of their lacunae become calcified. The thin walled, calcified lacunae are called primary areolae. Calcified cartilage is very heavily mineralised.

8) At the same time, cells deep within the perichondrium differentiate into osteoblasts and secrete a thin layer of bone called the periosteal collar around the central part of the cartilage shaft.

9) The perichondrium around the newly formed periosteal collar undergoes a transformation and becomes a periosteum, but it continues to provide a rich source of chondroblasts.

10) Connective tissues and blood vessels called osteogenic or periosteal buds 'sprout' from the periochondrium, through the periosteal collar and permeate the primary areolae of the calcified cartilage matrix.

11) Undifferentiated cells pass through the periosteal buds into the most central regions of the cartilage, where they differentiate into chondroclasts (large cells responsible for cartilage resorption) and begin to dissolve and resorb the calcified areolae walls, thus opening up large central cavities within the cartilage shaft.

12) Osteoprogenitor cells also migrate along the periosteal buds from the perichondrium to the centre of the shaft, where they differentiate into osteoblasts and commence osteoid secretion on the walls

of the ensuing spaces. Such deposition of bone within the centre of a cartilage diaphysis is called a primary ossification centre.

13) The replacement of cartilage with bone, through the action of chondroclasts and osteoblasts, continues along the cartilage shaft towards the epiphyses.

14) As endochondral ossification continues, the periosteal bone collar becomes thicker and lengthens through osteoblastic activity.

Endochondral ossification is a gradual process which begins in the central diaphyses of all limb bones and proceeds towards the epiphyseal ends, where cartilage proliferation continues; thus developing limb bones can show several stages of development. The following developmental stages / zones were isolated by Leeson *et al.* (1985) to illustrate the gradual and progressive nature of endochondral ossification:

1) Quiescent or Reserve Zone - this is located nearest to the ends of the bone and consists of primitive hyaline cartilage that shows growth in all directions.
2) Proliferation Zone - this is a highly active zone in which rows of chondroblasts can be seen. Each cell is separated from its neighbour by a thin wall of cartilage matrix. Growth occurs in a longitudinal manner through the addition of secretory cells at the distal end of each row.
3) Maturation Zone - here chondroblasts are trapped within their ensuing cartilage matrix and they transform into chondrocytes. Their enlargement adds to the length of the cartilage in this region.
4) Calcification Zone - here the cartilage matrix around the lacunae of the enlarged chondrocytes becomes calcified as minerals are deposited within it.
5) Retrogressive Zone - eventually the chondrocytes die and they and the thin walls of matrix between them are resorbed by chondroclasts (thicker walls of matrix between the actual rows of chondrocytes do temporarily survive as supporting structures) and the ensuing spaces are invaded by primary marrow tissue.
6) Ossification Zone - here the mesenchymal cells of the primary marrow tissue give rise to osteoblast cells which gather together on the remaining plates of calcified cartilage to form a primary ossification centre and commence osteoid secretion.

7) Resorption Zone - here ossification proceeds along the cartilage template and the internal marrow cavity is increased in size (to form the secondary marrow cavity) through the resorption of the inner surviving cartilage and the earliest deposited bone.

At around the time of birth additional ossification centres develop in the epiphyses. These are known as secondary ossification centres, and the sequence of bone development in these regions is as described above for the diaphysis, however in the epiphyses ossification spreads in a radial (not a longitudinal) manner. Ossification continues in the epiphyses until most cartilage is replaced by bone. Cartilage does persist in two regions:

1) Over the free ends of the epiphyses as articular cartilage (this persists throughout the life of a joint bearing surface).
2) Between the epiphysis and the diaphysis to form the epiphyseal plate or disc. These cartilaginous discs continue to grow between the diaphyses and the epiphyses (through chondroblastic activity), causing an overall increase in bone length. Once the full adult bone length is achieved the cartilaginous discs cease proliferation and are replaced by bone in the normal way and thus the epiphyses and diaphyses become united.

Epiphyseal ossification is a complex process and some bones can develop more than one centre of secondary ossification. For example, in humans the proximal epiphysis of the humerus is completely composed of cartilage at birth, but three centres of secondary ossification develop within it during childhood; one forms the articular surface of the epiphyses and the other two form the greater and lesser tubercles. All three expand outwards and fuse together before fusing with the diaphyses. The distal epiphysis of the humerus and of the femora also have multiple secondary ossification centres which fuse together before fusing to the main shaft.

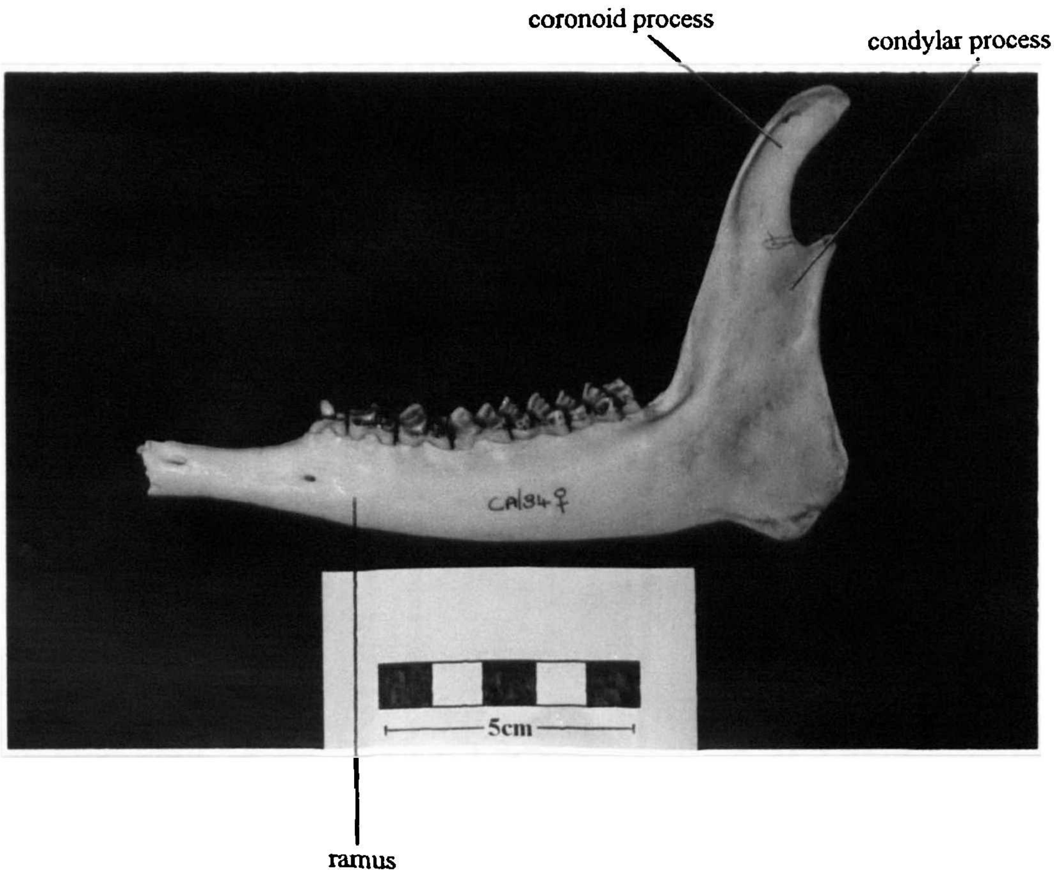
2.3.5 Mandibular Development

The bone that forms the main focus of the this study is the roe deer lower jawbone or 'mandible', and its development involves both intramembranous and endochondral ossification:

- The base or 'ramus', and main focus in this study, is formed through intramembranous ossification (Plate 2.3).
- The coronoid and condylar processes are formed through endochondral ossification (Plate 2.3).

The mandible originates from a condensation of mesenchymal cells which, early in embryonic life, divides into two equal parts or arches, one part becoming the maxillary (upper jaw) arch and the other the mandibular arch. The mesenchymal cells of the mandibular arch condense and proliferate to give rise to a cartilaginous rod called Meckel's cartilage. Around the same time secondary separate cartilaginous regions develop in the coronoid and condylar processes. Meckel's cartilage serves as an initial supporting template for the mandible but is resorbed prior to any development of bone, so the main body of the mandible forms through intramembranous ossification, but in the secondary cartilaginous regions bone development proceeds through endochondral ossification. These secondary centres of development eventually fuse with the main body of the mandible.

Plate 2.3: A Roe Deer Mandible



2.4 Bone Turnover (Remodelling)

All bone tissue laid down during endochondral or intramembranous ossification is woven. Its collagen fibres are deposited in irregular bundles and its osteocytes are numerous and appear randomly scattered. However, woven bone is a temporary tissue (above) and is at some time resorbed and replaced by the more highly organised lamellar bone tissue.

2.4.1 Woven To Lamellar Bone

For some unknown reason, and before growth has ceased, osteoblasts stop producing the coarse collagen fibre bundles of woven bone and begin to secrete the fine and highly orientated collagen fibres of lamellar bone. Lamellar bones consist of:

2.4.1.1 Circumferential Lamellae

2.4.1.2 Osteons

2.4.1.1 Circumferential Lamellae

These are deposited on periosteal bone margins or endosteal bone margins or both by osteoclasts during continued growth (in width and girth). Their deposition may follow or precede localised osteoclastic resorption of woven bone.

2.4.1.2 Osteons

There are three types of osteons:

- Primary Osteons
- Secondary Osteons
- Fragmentary Osteons

- **Primary Osteons**

  These are formed prior to osteoclastic resorption and result from the inward deposition of lamellae within the irregular vascular spaces of coarse woven bone. Lamellae deposition continues until the vascular space is reduced to a narrow canal containing blood vessels.

- **Secondary Osteons**

  These are formed after osteoclastic resorption of pre-existing woven bone. Osteoclasts erode bone in an eccentric manner, beginning on the walls of a vascular canal and 'eating' into the surrounding bone tissue. The tunnel of bone excavated by an osteoclast is called a cutting cone. Secondary bone is then deposited within the cutting cone by osteoblasts (to form a 'closing cone' (in the same manner as in the formation of primary osteons, that is lamellae are deposited inwardly within the space until it is reduced to a narrow canal which contains blood vessels). However, secondary osteons can be distinguished from primary osteons because they are delimited by a cement reversal line which marks the point where osteoclastic resorption ceased and osteoblastic lamellae deposition commenced. The balanced activity of osteoclastic bone resorption and osteoblastic bone formation, as witnessed during the formation of secondary osteons, is called 'remodelling'.

- **Fragmentary Osteons**

  These are the ~~partial~~ remains of primary and secondary osteons that are partially resorbed during remodelling.

2.4.2 The Continuous Turnover Of Lamellar Bone

Remodelling does not cease with the formation of secondary osteons but instead continues throughout life with successive generations of osteons replacing the earlier formed ones. Such internal remodelling, associated with continued turnover, should not be confused with growth remodelling, which is associated with gross overall changes in shape of a bone during growth. Whitfield & Morley (1995; p. 382) state that internal or osteonal remodelling is essential "to repair micro-fractures resulting from normal use" and "to maintain optimal load-bearing configurations", and Enlow (1990) recognises its importance in maintaining mineral homeostasis because it releases calcium and other mineral ions (locked in the skeleton) back into the blood stream during resorption.

In humans growth, and growth remodelling cease between sixteen and twenty years, but internal remodelling continues throughout life, with the skeleton being completely turned over every eight to ten years by 3×10^7 teams of osteoclasts and osteoblasts called Basic Multicellular Units (BMUs) (Frost, 1986). Initially, as with secondary osteon formation, osteoclasts are activated by some unknown signal(s) and begin to excavate an area of bone. Although the specific signal(s) which stimulates osteoclastic activity is unknown, the whole process of remodelling is known to be regulated by systemic polypeptide hormones, ~~such as polypetide~~ steroid and thyroid hormones and by local factors which cause the differentiation and activation of osteoclasts and osteoblasts. Osteoclasts can move along an area of bone up to 25μm long each day, and may continue to advance for up to eighteen days until they have excavated a cutting cone some 430μm long. Resorption results in the liberation of factors such as insulin-like growth factor 1 (IGF-1) and IGF-binding protein 5 (IGFBP 5), which were incorporated into bone by osteoblasts during its formation. When they are released during resorption they activate new osteoblasts to form new secondary osteons. Each complete Basic Multicellular Unit cycle (BMU) lasts between fifty and one hundred days. The rate of turnover slows with advancing age, but the degree of remodelling, as indicated by the area of circumferential lamellar bone and the numbers of primary, secondary and fragmentary osteons within an area of bone, can be used to estimate the age at death of skeletal material (Chapter 3).

2.5 In Conclusion

The development and turnover processes described above give rise to changes in the quantity and distribution of histological features within bone with increasing age. These features include:

- Non-Haversian canals - the large vascular spaces of woven bone.
- Primary osteons - formed through the infilling of the large vascular spaces within woven bone, as its structure is transformed to that of adult lamellar bone.
- Secondary osteons - formed through the continual turnover or remodelling of adult lamellar bone.
- Fragmentary osteons - also formed through the continual turnover or remodelling of adult lamellar bone.

- Periosteal circumferential lamellae - laid down around the outer circumference of cortical bones during expansions in girth.

The changing proportions of some of these features with respect to age in roe deer are shown in Plates 2.4 to 2.15. (Circumferential lamellae area showed no relationship to age in roe deer - Chapter 5).

In the next chapter the varying approaches to the quantification of these features in humans (and in a population of laboratory rats) and their use to estimate age are discussed. Comparisons between age estimates based on quantified bone histology and age estimates based on more traditional macroscopic techniques are also considered.

Plate 2.4: An SEM Image Of A Cross Section Of A Mandible (position 1, see Figure 4.1) From A Roe Doe Kid.

The whole cortex consists of woven bone, the large vascular spaces of which have recently been infilled with concentric lamellae to form primary osteons.

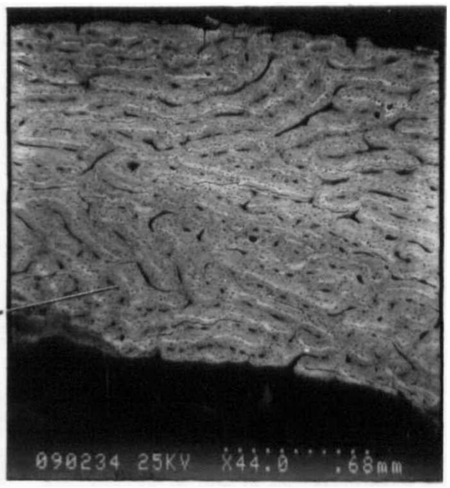


Plate 2.5: An SEM Image Of A Cross Section Of A Mandible (position 1, see Figure 4.1) From An 8 Year Old Roe Doe.

The outer cortex consists of circumferential lamellae and woven bone, in which the large vascular have been infilled with concentric lamellae. The inner cortex consists of bone that has undergone secondary remodelling, and both secondary and fragmentary osteons can be seen.

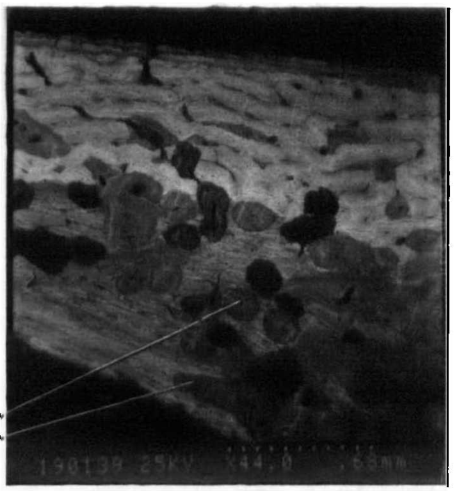


Plate 2. 6: An SEM Image Of A Cross Section Of A Mandible (position 1, see Figure 4.1) From An 11 Year Old Roe Doe.

Secondary remodelling can be seen to have extended from the inner, deeper regions of the cortex to the outer cortical regions.

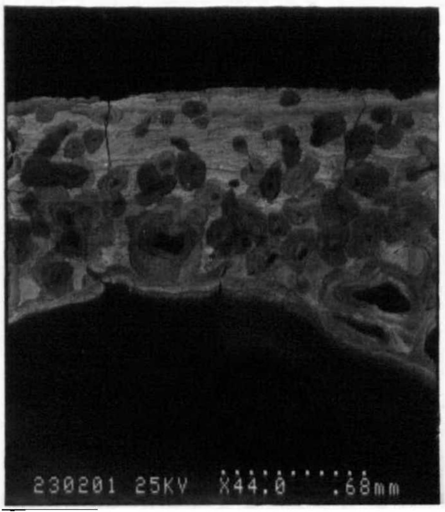
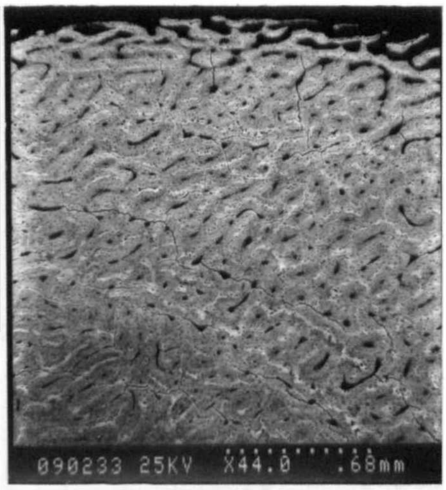
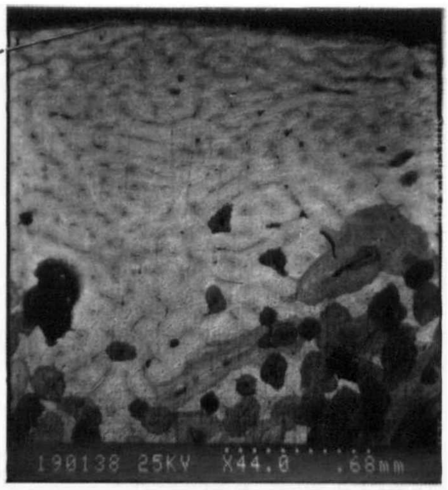


Plate 2.7: An SEM Image Of A Cross Section Of A Mandible (position 2, see Figure 4.1) From A Roe Doe Kid.



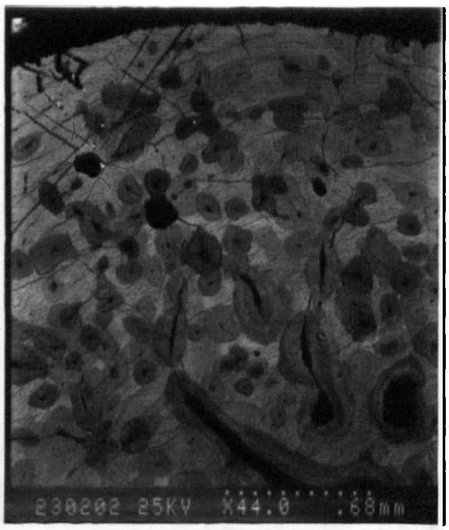
Again, the whole cortex consists of woven bone, the large vascular spaces of which have recently been infilled with concentric lamellae to form primary osteons.

Plate 2.8: An SEM Image Of A Cross Section Of A Mandible (position 2, see Figure 4.1) From An 8 Year Old Roe Doe.



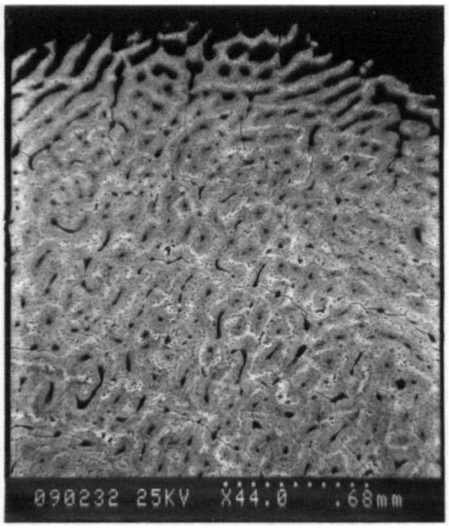
Circumferential lamellae have been damaged, during processing (Chapter 4), but much of the cortex can be seen to consist of woven bone, in which the large vascular have been infilled with concentric lamellae. Only the deepest regions of the inner cortex consists of bone that has undergone secondary remodelling.

Plate 2. 9: An SEM Image Of A Cross Section Of A Mandible (position 2, see Figure 4.1) From An 11 Year Old Roe Doe.



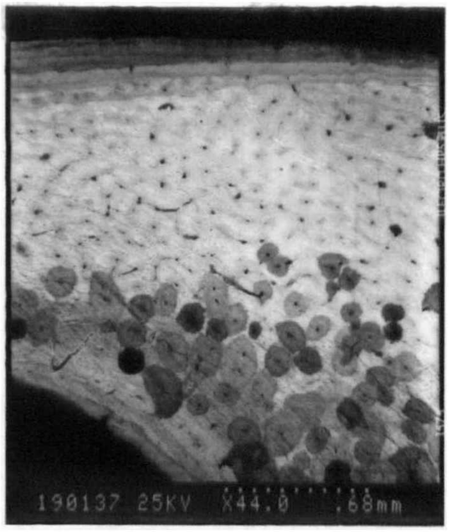
Again, secondary remodelling has extended throughout much of the inner and outer cortex.

Plate 2.10: An SEM Image Of A Cross Section Of A Mandible (position 3, see Figure 4.1) From A Roe Doe Kid.



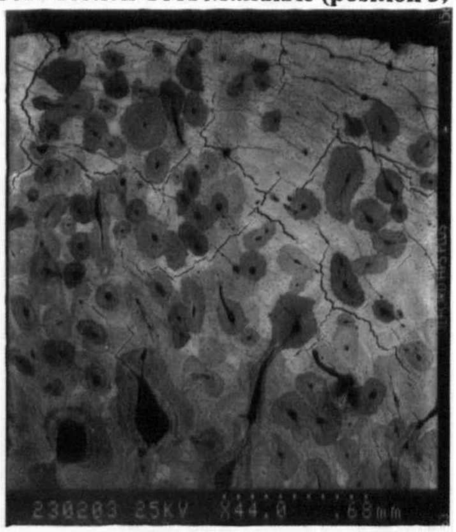
Again, the whole cortex consists of woven bone, the large vascular spaces of which have recently been infilled with concentric lamellae to form primary osteons.

Plate 2.11: An SEM Image Of A Cross Section Of A Mandible (position 3, see Figure 4.1) From An 8 Year Old Roe Doe.



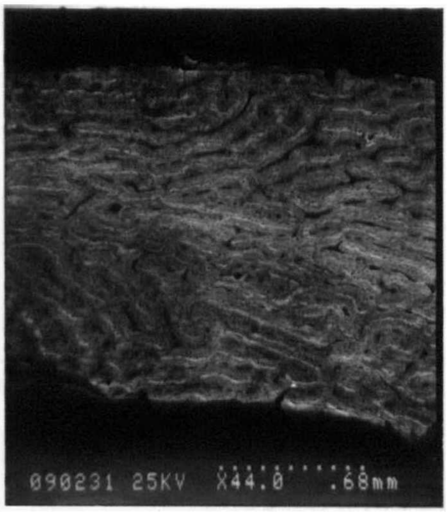
Intact circumferential lamellae can be seen. The outer part of the cortex, again, consists of woven bone in which the large vascular spaces have recently been infilled with concentric lamellae, and the inner cortex consists of bone that has undergone secondary remodelling.

Plate 2. 12: An SEM Image Of A Cross Section Of A Mandible (position 3, see Figure 4.1) From An 11 Year Old Roe Doe.



Again, secondary remodelling has extended throughout much of the inner and outer cortex.

Plate 2.13: An SEM Image Of A Cross Section Of A Mandible (position 4, see Figure 4.1) From A Roe Doe Kid.

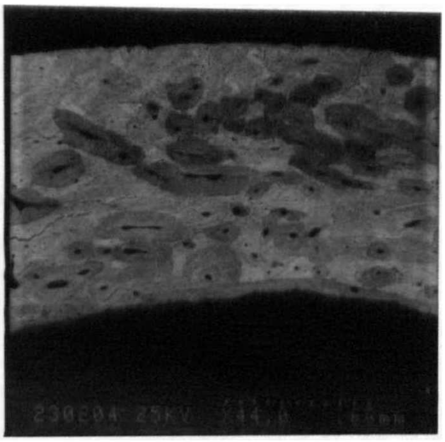


Again, the whole cortex consists of woven bone, the large vascular spaces of which have recently been infilled with concentric lamellae to form primary osteons.

Plate 2.14: An SEM Image Of A Cross Section Of A Mandible (position 4, see Figure 4.1) From An 8 Year Old Roe Doe.

Some circumferential lamellae can be seen, and secondary remodelling appears to have extended throughout the cortex. There is some remaining woven bone, in which the large vascular spaces have recently been infilled with concentric lamellae.

Plate 2. 14: An SEM Image Of A Cross Section Of A Mandible (position 4, see Figure 4.1) From An 11 Year Old Roe Doe.



Again, secondary remodelling has extended throughout much of the inner and outer cortex.

CHAPTER 3

BONE HISTOLOGY-BASED AGE DETERMINATION

3.1 Microscopic Age Determination

Since 1965 there has been an increasing interest in the use of bone microstructure as the basis for determining age at death in humans and rats, based on the following studies:

3.1.1 Kerley (1965) and Kerley & Ubelaker (1978)

3.1.2 Ahlqvist & Damsten (1969)

3.1.3 Singh & Gunberg (1970; 1971) and Singh *et al.* (1974)

3.1.4 Thompson (1979)

3.1.5 Samson & Branigan (1987)

3.1.6 Ericksen (1991)

3.1.7 Stout & Paine (1992)

3.1.8 Wallin *et al.* (1994)

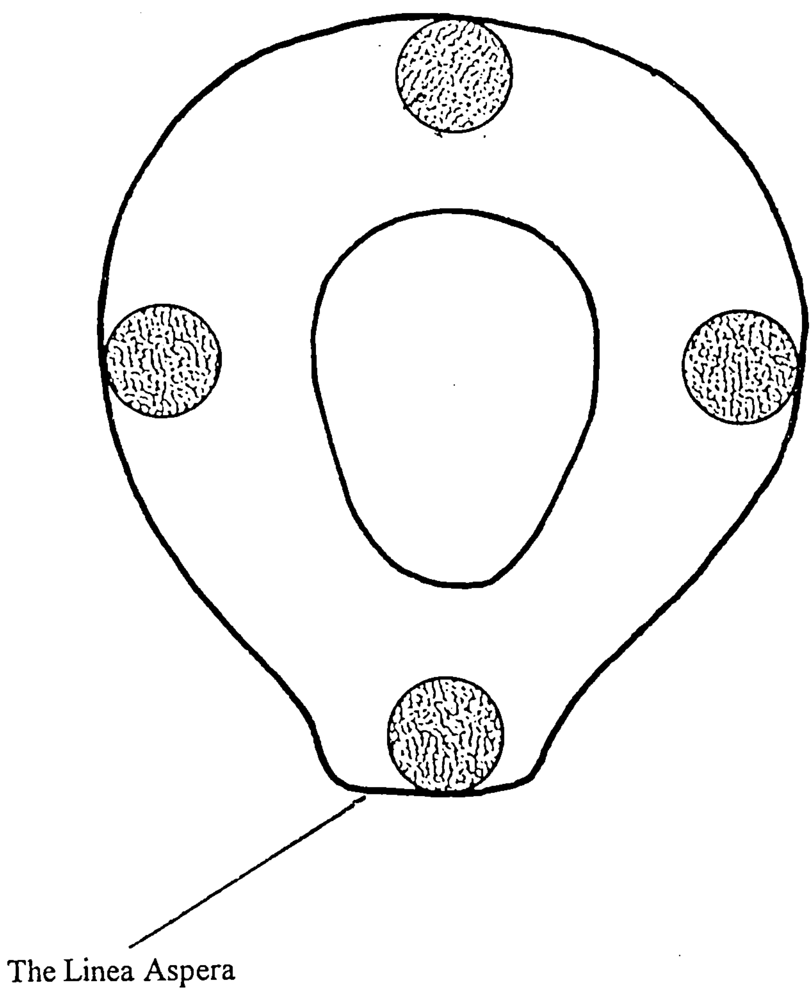
3.1.1 KERLEY (1965) AND KERLEY & UBELAKER (1978)

Kerley was the first to establish a system of age determination based upon counts of histological features in cortical bone. His work developed out of findings that there were 'microscopic changes in structure that occurred at the midshaft of the femur as age increased' (Amprino & Bairati, 1936, cited in Kerley 1965; Jowsey, 1960; Frost, 1964; Enlow, 1968), and in response to the failings of macroscopic age determination techniques which he described as 'limited in application by the condition of the remains and in accuracy by the age of the individual'.

Ground sections were prepared (the preparation technique was not detailed) from eighty-eight men and twenty-nine women ranging in age at death from new born to ninety-five years, and Kerley determined the numbers of:

- Complete secondary osteons - These were seen to be "easily recognisable in cross-section as a vascular canal surrounded by concentric lamellae, which contain rather evenly spaced osteocytes

Figure 3.1: Enlarged Cross Section Of A Human Femur Showing The Location Of The Four Circular Fields Of View Used By Kerley (1965)



in their lacunae. Around the entire periphery of the osteon there is a reversal line that marks the area where osteoclastic resorption stopped and was followed by new bone formation" (Kerley; 1965, p. 151). They were only recorded if their central canal was untouched by remodelling and if they judged to be 'more than 80% complete'.

- Fragmentary osteons - Kerley (1965, p. 151) described their formation: "As osteoclasts burrow channels through Haversian bone, fragments of old osteons may surround the edge of the channel and remain after resorption ceases and replacement begins."
- Circumferential lamellar bone - This was seen to be "composed of evenly spaced bands, or lamellae, that run parallel to each other around the outer part of the cortex" (Kerley; 1965, p. 151).
- Non-Haversian canals - This count included 'all primary vascular canals and those that had been infilled with concentric lamellae to form primary osteons or pseudo-Haversian systems'.

It is noted that Kerley is one of the few to described the histological features selected for recording, and the terms he used are in line with the description of bone formation presented in Chapter 1. The features were recorded in each of four anatomically defined fields (Figure 3.1) in 126 mid-shaft ground cross-sections of the femur, tibia and fibula. Fields were located in the outer peripheral areas of the cortex as "preliminary microscopic examination ... revealed that the structural changes normally associated with age seemed better spaced to cover the total life span in the outer third of the cortex than in the middle or inner thirds" (Kerley; 1965, 151). Each field measured 1.25mm in diameter and was examined at x100 with a polarising light microscope. The numbers of non-Haversian canals in all four fields were totalled to provide a single non-Haversian count for each specimen. The same procedure was adopted for secondary osteon and fragmentary osteon counts, but the percentage of circumferential lamellar bone area was averaged rather than totalled to provide a single value for each specimen. Composite values of the histological structures in all four fields were regressed (linear and curvilinear regressions were conducted as appropriate) against documented age (Table 3.1). The pattern of bone turnover for all three bones studied was the same:

- Secondary osteon number increased with age.
- Fragmentary osteon number increased with age.

Table 3.1: Kerley's (1965) Regression Formulae For Estimating Age (y) In Years From Various Histological Counts (x)

Histological Count (x)	**Regression**	**Correlation***	**Standard Error**
Femoral osteons	$y= 3.473+0.144x+0.003x^2$	0.922	± 9.39
Femoral fragments	$y= 8.786+0.834x$	0.864	± 12.19
Femoral lamellae	$y=79.455-2.427x+0.023x^2$	-0.870	± 11.78
Femoral non-haversian	$y=57.811-1.728x+0.013x^2$	-0.815	± 13.85
Tibial osteons	$y=-10.082+0.634x$	0.925	± 6.69
Tibial fragments	$y=-7.061+0.931x+2.210x^2-2.538x^3$	0.947	± 7.78
Tibial lamellae	$y=76.338-1.794x+0.011x^2$	-0.816	± 13.62
Tibial non-haversian	$y=70.270-0.944x+0.647x^2-0.011x^3$	-0.790	± 9.63
Fibular osteons	$y=2.366-0.538x+0.018x^2-0.001x^3$	0.922	± 8.83
Fibular fragments	$y=1.328-0.058x+0.034x^2$	0.974	± 5.27
Fibular lamellae	$y=69.108-2.208x+0.015x^2$	-0.881	± 10.85
Fibular non-haversian	$y=55.241-4.300x+0.050x^2$	-0.879	± 10.70

* significance level not stated

Table 3.2: Kerley & Ubelaker (1978) Revised Regression Formulae For Estimating Age (y) In Years From Various Histological Counts (x)

Histological Count	**Regression**
Femoral osteons	$y= 2.278+0.187x+0.00226x^2$
Femoral fragments	$y=5.241+0.509x+0.017x^2-0.00015x^3$
Femoral lamellae	$y=75.017-1.790x+0.0114x^2$
Femoral non-haversian	$y=58.390-3.184x+0.0628x^2-0.00036x^3$
Tibial osteons	$y=-13.4218+0.660x$
Tibial fragments	$y=-26.997+2.501x-0.014x^2$
Tibial lamellae	$y=80.934-2.281x+0.019x^2$
Tibial non-haversian	$y=67.872-9.070x+0.440x^2-0.0062x^3$
Fibular osteons	$y=-23.59+0.74511x$
Fibular fragments	$y=-9.89+1.064x$
Fibular lamellae	$y=124.09-10.92x+0.3723x^2-0.00412x^3$
Fibular non-haversian	$y=62.33-9.776x+0.5502x^{*}-0.00704x^3$

*[*Sic;* should read $0.5502x^2$]

- Circumferential lamellar bone area decreased with advancing age, and was absent in many of the older individuals.
- Non-Haversian canals were more frequent in juveniles than adults, and were absent in all those over fifty-five years.

Statistical analysis revealed that:

- All four feature counts were highly correlated with age for all three bones.
- For the tibia and fibula, secondary and fragmentary osteon numbers showed stronger correlations with age than either non-Haversian canal number or circumferential lamellar bone area.
- For the femur, osteon number and circumferential lamellar bone area showed stronger correlations with age than fragmentary osteon number and non-Haversian canal number.
- The strongest correlation with age was shown by fibula fragmentary osteon number.
- When two standard errors of the estimate were used as the limits of confidence, the regression equation derived from the count of fibula fragmentary osteons allowed age to be estimated to within ±10 years of actual age 95% of the time. The actual standard error for this regression equation was ± 5.27 years.

Although untested, Kerley assumed that sex and racial affinity did not affect the results.

The influence of disease processes on cortical remodelling was also explored through the examination of an additional seventy-eight sections from the femora of individuals who had experienced differing pathologies including trauma, inflammation, cardiovascular irregularity, atrophy, neoplasm and metabolic disorder. Fields away from obvious centres of pathology were analysed. Only in one individual with Paget's disease was the reliability of the histological age estimation technique disrupted and 'an excessive number of fragmentary osteons' noted. In microscopic fields that exhibited gross pathological lesions age changes could not, in any case, be accurately assessed. The histological age estimation technique was also applied to a series of archaeological specimens. These ranged in date from five hundred to five thousand years B.P., and came from a variety of burial conditions and exhibited differing degrees of preservation, mineralisation and leaching - however, all showed sufficient microstructural clarity for histological quantifications to be conducted.

Some years later, Kerley & Ubelaker (1978) published several corrections to the method. They reported that the original microscopic field size of 1.25mm stated by Kerley (1965) was incorrect, and that it should have been quoted as 1.62mm. They also presented new regression formulae for estimating age derived from counts of tibial fragmentary osteons and fibula osteons (Table 3.2), because those given by Kerley (1965) were found to contain errors which resulted in unreasonable age estimates.

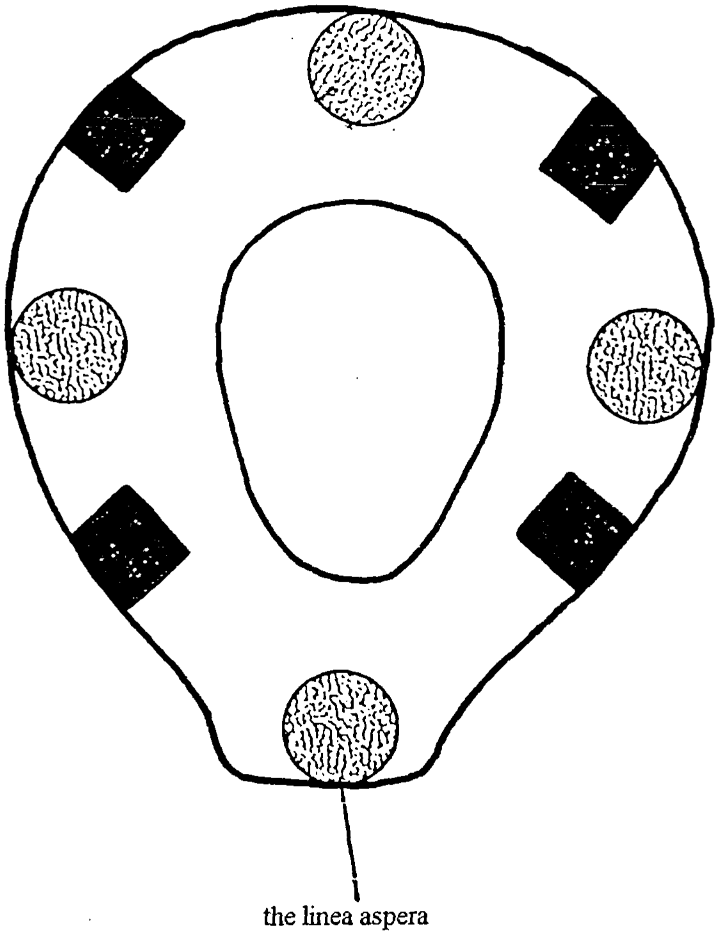
3.1.2 AHLQVIST & DAMSTEN (1969)

Ahlqvist & Damsten highlighted three practical problems with Kerley's age estimation technique, and proposed several modifications:

1. Identification and Counting of Histological Features - Difficulty was experienced in distinguishing osteons (no distinction between primary and secondary osteons was mentioned) from fragmentary osteons using the Kerley '80% complete' criterion . Instead the average percentage area occupied by both secondary and fragmentary osteons in four fields per sample was calculated and regressed against known age. Circumferential lamellar bone area and non-Haversian canal number were not recorded: it was noted that as these two features decreased in quantity with age, at the expense of increasing numbers of secondary and fragmentary osteons, then a count of either pair of increasing or decreasing features was all that was required to determine their proportionate percentage ratios.

2. Microscopic Field Shape - A square field of view was used, rather than Kerley's circular field. They reported that "the main alterations in the percentage of secondary osteons and osteon fragments occur in the immediate subperiosteal parts of the bone" and that "squares cover these areas better than circular fields" (Ahlqvist & Damsten; 1969, p. 208).

Figure 3.2: The Location (avoiding the linea aspera) Of The Four Square Fields Of View Preferred by Ahlqvist & Damsten (1969) To Kerley's (1965) Circular Fields



3. Microscopic Field Location - When examining femoral sections, one field location selected by Kerley fell within the area of the linea aspera - this is a prominent tuberosity which runs down the caudal ('back') aspect of the femur and an area "where greater variation in osteon and fragmentary osteon number not correlated to age" was noted (Ahlqvist & Damsten; 1969, 208). Instead four fields of view adjacent to those of Kerley and away from the linea aspera were selected (Figure 3.2).

The modified histological age estimation technique was applied to twenty femoral midshaft ground cross-sections from cadavers of known age at death. Statistical analysis revealed that there was a strong correlation between the average percentage field area occupied by secondary and fragmentary osteons and age, and the regression equation for estimating age derived from the percentage area count of femoral secondary and fragmentary osteons was found to have a standard error of ± 6.71 years (Table 3.3). The Ahlqvist & Damsten standard error of estimate was greater than those derived by Kerley (above) for fibula fragmentary osteon and tibia secondary osteon counts, but was less than those derived for femoral counts.

Table 3.3: Ahlqvist & Damsten's (1969) Regression Of Percentage Area Of Femoral Secondary And Fragmentary Osteons (x) On age (y) In Years

Histological Count	Regression	Correlation	Standard Error
Percentage secondary and fragmentary osteons	$y=0.991x-4.96$	0.965*	± 6.71 years

* significance level not stated

Bouvier & Ubelaker (1977) carried out an independent comparison of both the Kerley (1965) and Ahlqvist & Damsten (1969) histological age determination techniques. They selected forty of Kerley's original sixty-seven femoral sections for the test. From these, they recorded the percentage of secondary and fragmentary osteons in the same four fields per section as Ahlqvist & Damsten, and converted these to age estimates using Ahlqvist & Damsten's regression equation (Table 3.3). Similarly, they recorded the number of secondary osteons in the same four fields per section as Kerley, and converted these to age estimates using Kerley's femoral secondary osteon regression equation (Table 3.1). The features were scored and counted without difficulty, but a degree of inter-observer error was noted in the 'Kerley-based' counts of secondary osteons in microscopic fields located on the linea aspera, and (like Ahlqvist & Damsten) it was concluded that it was more difficult to obtain a reliable count from this field. The resulting age estimates were compared with actual ages, and the standard error of each regression equation when used to determine age was calculated separately for this study and compared with the original standard errors:

- Kerley-based age estimates differed from known age by an average of ± 8.2 years.
- Ahlqvist & Damsten-based age estimates differed from known age by an average of ± 9.5 years.
- The standard error of the estimate calculated for the Kerley regression equation in this study (although not stated) did not differ significantly from that originally reported by Kerley.
- The standard error of the estimate calculated for the Ahlqvist & Damsten regression equation in this study was ± 11.65 years, much larger than the ± 6.71 years originally reported.

It was noted that the Kerley technique was developed on a sample size of sixty-seven and that the Ahlqvist & Damsten technique was developed on a sample size of twenty. The increased standard error of the Ahlqvist & Damsten technique is probably wholly accounted for by a larger range of individuals being included. The accuracy of the two ageing techniques was assessed by calculating the average difference of estimated age from true age for two age groups (Table 3.4).

Table 3.4: Accuracy Of The Kerley (1965) And Ahlqvist & Damsten (1969) Histology-Based Age Determination Techniques For Ageing Those 45 Years Or Under And Those Over 45

Replicated Technique	Age Group	Average difference from actual age
Kerley (1965)	20 to 45	± 9.93 years
Kerley (1965)	46 to 90	± 8.05 years
Ahlqvist & Damsten (1969)	20 to 45	± 13.87 years
Ahlqvist & Damsten (1969)	46 to 90	± 5.70 years

The Kerley technique was found to be a fairly consistent performer for those aged both under and over forty-five years, whereas the Ahlqvist & Damsten technique was found to be much more accurate for those over forty-five years than for those forty-five years or under. It was noted that

Kerley's technique was developed on an original sample of even age spread, with an average age of 41.55 years, but Ahlqvist & Damsten's original sample had an average age at death of 55.45 years. This might explain the greater level of accuracy in the over forty-five years age group. Again, it was found possible to record the counts and measurements, but a higher level of inter-observer error was noted in the 'Kerley-based' counts of secondary osteons in the fields located on the linea aspera. In summary Bouvier & Ubelaker found that:

- The Kerley age determination technique (number of secondary osteons in the femur only) was soundly based on a sample of even age distribution and of adequate size.
- The smaller size and uneven age distribution of Ahlqvist & Damsten's original study sample affected the accuracy of subsequent age estimations based upon their technique.

Stout & Gehlert (1980) similarly compared the revised Kerley technique (Kerley & Ubelaker, 1978) with the Ahlqvist & Damsten technique on an independent sample. They found that average ages derived from the Kerley & Ubelaker regression equations produced the most accurate and reliable estimates, although the Ahlqvist & Damsten technique gave good results for those > 60 years. In a later study, Stout & Gehlert (1982) recognised the importance of replicating Kerley's revised field size of 1.62mm in diameter (Kerley & Ubelaker, 1978) when using his technique. Stout & Stanley (1991) also expressed a preference for the Kerley secondary osteon count age determination technique, and found it to have overall greater accuracy than the Ahlqvist & Damsten percent osteonal bone method.

3.1.3 SINGH & GUNBERG (1970,1971) AND SINGH *et al.* (1974)

Both Kerley and Ahlqvist & Damsten had confined their studies to the lower limb bones but Singh & Gunberg (1970) investigated age related cortical remodelling in the human mandible as well as the femur and tibia. Bone fragments (1cm^2) were removed from the posterior border of the mandibular ramus and from the midshaft of the anterior surface of the femora and tibiae of thirty-three men. These were then fixed and decalcified in formalin- formic acid, paraffin embedded and cut into 10μm thick cross-sections for slide mounting. Four slides per bone fragment were prepared, and

Table 3.5: Singh & Gunberg's (1970) Regression Formulae For Estimating Age (y) In Years From Various Histological Counts (x)

Histological Count	Regression	Multiple R^1	Standard Error
Mandible			
I	$y=20.82+0.85x_1+0.87x_2-0.22x_3$	0.979	± 2.55
II	$y=-18.99+.13x_1+1.76x_2$	0.976	± 2.69
III	$y=32.23-0.92x_1-0.30x_3$	0.978	± 2.58
IV	$y=74.73+1.52x_2-0.45x_3$	0.969	± 3.04
V	$y=-28.24+1.68x_1$	0.969	± 3.02
VI	$y=5.31+5.00x_2$	0.950	± 3.83
VII	$y=103.99-0.63x_3$	0.966	± 3.16
Femur			
I	$y=27.65+0.65x_1+0.78x_2-0.26x_3$	0.958	± 3.24
II	$y=-14.69+1.13x_1+1.11x_2$	0.948	± 3.55
III	$y=29.59+0.79x_1-0.28x_3$	0.957	± 3.25
IV	$y=61.25+1.74x_2-0.44x_3$	0.949	± 3.52
V	$y=16.10+1.38x_1$	0.945	± 3.60
VI	$y=2.00+5.16x_2$	0.889	± 5.01
VII	$y=89.01-0.62x_3$	0.937	± 3.82
Tibia			
I	$y=43.52+0.291x_1+1.47x_2-0.34x_3$	0.964	± 3.02
II	$y=-3.40+0.67x_1+2.27x_2$	0.936	± 3.93
III	$y=48.61+0.53x_1-0.38x_3$	0.957	± 3.22
IV	$y=54.79+2.19x_2-0.4x_3$	0.960	± 3.12
V	$y=-4.76+1.15x_1$	0.919	± 4.33
VI	$y=5.10+4.88x_2$	0.908	± 4.59
VII	$y=91.32-0.64x_3$	0.935	± 3.88

<u>Key</u>

Multiple R^1: Squared correlation coefficient, at 5% level of significance.

I: x1, x2, x3	V: x1	x1: Total number of osteons in two fields
II: x1, x2	VI: x2	x2: Average number of lamellae per osteon
III: x1, x3	VII: x3	x3: Average diameter of Haversian canal (μm)
IV: x2, x3		

between one and three adjacent sections were mounted on each slide. One slide per fragment was stained with carbol-thionin for examination. Additional mandibular fragments were removed from a further nineteen men whose femora and tibiae were not available for analysis, and these were dehydrated and embedded in methylmethacrylate before being cut with a rotary saw to produce a cross-section between 150μm and 200μm thick. These sections were then hand ground to a thickness of between 30μm and 50μm, and slide mounted. Selected slides were analysed at a magnification of x100, which allowed circular fields of view 2mm in diameter to be studied. The following counts or measurements were recorded for each section:

1) The total number of osteons (in which the complete central Haversian canal was visible) in two fields, each located in the periosteal third of the bone cortex.
2) The average number of lamellae per complete secondary osteon identified in count 1.
3) The average Haversian canal diameter of each complete secondary osteon identified in count 1.

Non-Haversian canals and circumferential lamellar bone area were not recorded, because most bone fragments had been removed from individuals aged over forty years and Kerley had reported that these features were rarely encountered in this age group.

All measurements were correlated with known age and linear regression models were fitted. For each bone the measurements were regressed against age on their own, with each of the other features in turn, and altogether as a multiple regression (Table 3.5). Statistical analysis revealed that:

- In all bones osteon number increased with age.
- In all bones the average number of lamellae per osteon increased with age.
- In all bones Haversian canal diameter decreased with age.
- For all three bones there was a high degree of correlation between secondary osteon number and age, but this was strongest for the mandible.
- Mandible counts had the smallest standard error of estimate for every combination of measurements regressed on age. The standard error was most reduced when all three

measurements were regressed on age and greatest when age estimates were based solely on the number of lamellae per osteon.

Resulting regression equations were used to age bones removed from seven women, and "for the limited material available, significant sex differences were not noted" (Singh & Gunberg; 1970, p. 378).

Singh & Gunberg (1971) further investigated age related cortical remodelling in laboratory rats. Thirty-five female Sprague-Dawley rats were sacrificed in groups of five at two, twenty-five, thirty, sixty, ninety, one hundred and one hundred and twenty days. Decalcified cross-sections of cortical bone from the mandible, femur and tibia of each rat were prepared and analysed as in their earlier study (Singh & Gunberg, 1970) but with the inclusion of an additional measurement - a count of primary non-Haversian canals - a prominent feature of rat cortical bone. Again, measurements were correlated with and regressed against age (Table 3.6) and a similar pattern of age-related bone turnover to that noted in humans was identified in the rat sample:

- In all three bones osteon number (although low) increased with age.
- In all bones the average number of lamellae per osteon also increased with age.
- All features showed strong correlations with age in all bones studied, but the strongest correlations were generally provided by counts taken from mandible fragments.

The additional measurement of the average number of primary non-Haversian canals in two fields revealed, as for Kerley (1965), that their number decreased with age and a strong negative correlation between age and this measurement was identified for all three bones studied. Finally, in 95% of all samples regression equations derived from counts of histological features allowed age to be estimated to within ± 3 days of its true value. The replicability of the measurement technique was checked by a second observer, who recounted 10% of all samples. Minimal inter-observer error was identified.

The work of Singh & Gunberg (1970; 1971) is encouraging. Histology based ageing was used to predict the ages of both human and rat study groups from small fragments of bone removed from the

Table 3.6: Singh & Gunberg's (1971) Regression Formulae For Estimating Log Age (y) In days In Rats From Various Histological Counts (x)

Histological Count	Regression	Multiple R[1]	Standard Error
Mandible			
I	$y=0.55-0.002x_1+0.48x_2+0.05x_3+0.001x_4+0.001x_5-0.05x_6-0.003x_7-0.005x_8$	0.982	± 0.125
II	$y=1.51+0.07x_1-0.07x_2-0.02x_3-0.10x_4$	0.974	± 0.139
III	$y=-1.17+0.21x_1-0.17x_2$	0.940	± 0.204
IV	$y=0.735+0.09x_1-0.10x_3$	0.947	± 0.192
V	$y=1.40+0.06x_1-0.10x_4$	0.973	± 0.137
VI	$y=2.25+0.10x_2-0.17x_3$	0.936	± 0.210
VII	$y=2.22+0.11x_2-0.13x_4$	0.967	± 0.151
VIII	$y=2.76-0.06x_3-0.11x_4$	0.970	± 0.145
Femur			
I	$y=3.0-0.13x_1+0.65x_2-0.34x_3+0.10x_4+0.003x_5-0.07x_6+0.01x_7-0.07x_8$	0.984	± 0.117
II	$y=3.79-0.04x_1+0.02x_2-0.16x_3-0.08x_4$	0.965	± 0.163
III	$y=-0.94+0.16x_1+0.01x_2$	0.906	± 0.253
IV	$y=2.73+0.01x_1-0.22x_3$	0.935	± 0.212
V	$y=1.50+0.06x_1-0.10x_4$	0.952	± 0.184
VI	$y=2.75+0.05x_2-0.21x_3$	0.936	± 0.211
VII	$y=2.17+0.12x_2-0.11x_4$	0.946	± 0.194
VIII	$y=2.99-0.12x_3-0.08x_4$	0.963	± 0.160
Tibia			
I	$y=4.13-0.11x_1+0.16x_2-0.36x_3+0.05x_4-0.002x_5-0.02x_6+0.02x_7-0.01x_8$	0.976	± 0.146
II	$y=4.55-0.06x_1-0.06x_2-0.14x_3-0.12x_4$	0.973	± 0.142
III	$y=-0.65+0.16x_1+0.03x_2$	0.928	± 0.223
IV	$y=2.85+0.01x_1-0.25x_3$	0.958	± 0.172
V	$y=3.69-0.04x_1-0.17x_4$	0.967	± 0.152
VI	$y=3.58-0.08x_2-0.30x_3$	0.958	± 0.171
VII	$y=3.04-0.03x_2-0.15x_4$	0.966	± 0.155
VIII	$y=3.01-0.10x_3-0.09x_4$	0.969	± 0.147

Key to Table 3.6

Multiple R^1: Squared correlation coefficient, at 5% level of significance.

I: x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8

II: x_1, x_2, x_3, x_4

III: x_1, x_2

IV: x_1, x_3

V: x_1, x_4

VI: x_2, x_3

VII: x_2, x_4

VIII: x_3, x_4

x_1: Total number of osteons in two fields.

x_2: Average number of lamellae per osteon.

x_3: Average diameter of Haversian canal (μm).

x_4: Total number of non-Haversian longitudinal canals in two fields.

x_5: Total number of osteons in two fields squared.

x_6: Average number of lamellae per osteon squared.

x_7: Average diameter of Haversian canal (μm) squared.

x_8: Total number of non-Haversian longitudinal canals in two fields squared.

Table 3.7: Major Bone Types Identified In Various Mammalian Orders By Singh *et al.* (1974)

Order	Sample Nos.	Bone Type	Comments
Rodentia	10	mostly PV-LC, some SH, NV	Sprague Dawley rats, 90 days old.
Marsupialia	1	PV-LC, some NV	Opossum, sex and age unknown.
Insectivora	2	PV-LC, some NV	1 female Haitian solenodon, age unknown. 1 male common tenrec, mature.
Artiodactyla	3	PV-RETIC, some NV	1 female mature blue duiker. 1 day old Indian sambar deer. 1 day old Reindeer.
Canidae	1	PV-LC	1 female fox.

<u>Key to Bone Types</u>

PV-LC: 'Primary Vascular with Longitudinal Canals' (primary osteons).

SH: 'Secondary Haversian' (secondary osteons).

NV: 'Non-Vascular' (no blood vessels are present).

RETIC: 'Reticular' (blood vessels form an unorganised reticulum throughout the bone and no predominant alignment may be distinguished / woven bone).

mandible as well as the femora and tibia. Further studies of histology based ageing in non-human populations are rare, and the lack of such studies may be attributed to the scarcity of known age animal populations on which original histological assessments may be made. However, Singh *et al.* (1974) were able to conduct a limited semi-quantitative survey of bone turnover in a variety of animal orders including primates, rodents, marsupials, insectivores, artiodactyls and carnivores from specimens obtained from the Bronx Zoological Gardens. Midshaft bone fragments were removed as available from ribs, tibiae and femora and prepared for microscopic analysis (Singh *et al.*; 1974, p. 422). Qualitative descriptions of the predominant bone types observed in fields in the periosteal, endosteal and middle thirds of each cross-section were made (Table 3.7) and measurements were taken of:

1) The average number of primary longitudinal canals per unit area of bone.
2) The average diameter (in μm) of the primary longitudinal canals.
3) The average number of lacunae per field.
4) The percentage of 'apparently empty' lacunae (no definition of this feature was given and it should be noted that section preparation can often result in the destruction of delicate cells housed within lacunae).
5) The presence or absence of zones of periosteal and endosteal lamellae.
6) The average number of osteons.
7) The average number of lamellae per osteon.
8) The average Haversian canal diameter of the osteons.

Unfortunately histological development could not be compared to age (or sex) as such information was lacking and sample sizes were limited, but several observations were made:

- Secondary osteons were not generally seen in non-primate species although rats did show 'a few scattered osteon-like structures'.
- Where present, osteons were most often seen in the endosteum than the periosteum.
- Marsupials, insectivores, artiodactyls and carnivores did not show any osteons - this was in contrast to Enlow (1968) who had noted secondary osteons in both artiodactyls and carnivores.

Singh *et al.* recognised that the specimens examined in this study may have been too young to develop secondary osteons and that Enlow had only seen secondary osteons in older artiodactyl and carnivore specimens.

- Most species showed some highly vascular bone with primary longitudinal canals.
- All specimens showed areas of acellular and non-vascular bone.
- "Species differences in bone microstructure involve the relative distribution of the same basic components which lend themselves to quantitative treatment." (Singh *et al.*, 1974).

This study showed that age-related histological features could be identified and were open to quantification in the bones of a variety of non-human species. These findings were extremely encouraging, and formed one of the main starting points of the current project.

3.1.4 THOMPSON (1979)

Thompson (1979) reduced the physical damage caused to skeletons through the removal of complete cross-sections for microscopic age determination (Kerley, 1965; Kerley & Ubelaker, 1978; Ahlqvist & Damsten, 1969) by developing a technique that required the analysis of only a small cortical core. A specially constructed drill was used to remove four hundred and twenty-nine mid-shaft cores (0.4cm in diameter) from the left and right femora and tibiae, as available, from sixty-four men and fifty-two women. An additional one hundred and twenty-two cores were also removed from the left and right humeri and ulnas of thirty-one individuals. Prior to section preparation, several non-histological variables were recorded for each of the cores:

- The wet weight - this was determined to the nearest 0.0001g by using a Merrler Model H207 balance.
- Cortical thickness - this was determined to the nearest 0.05mm after adherent marrow and the periosteum had been removed.
- Diameter and length - these were determined to the nearest 0.05mm.
- Wet bone density - this was estimated (in g/cm^3) by dividing the volume of the core by the wet weight of the core.

Table 3.8: Thompson's (1979) Regression Formulae For Estimating Age (y) In Years From Secondary Osteon Area (x)

Bone	N	Regression Equation	MCC	CoD	SEE
All left femora	91	y=6.677+101.936x	0.7734	0.5982	8.6455
All right femora	113	y=12.409+91.936x	0.7887	0.6221	7.8789
All left tibiae	112	y=20.835+82.235x	0.7036	0.4950	9.5163
All right tibiae	113	y=20.632+82.475x	0.7441	0.5537	8.6822
Male left femora	53	y=8.387+100.133x	0.7873	0.6199	8.2167
Male right femora	63	y=18.413+84.646x	0.8061	0.6499	7.0675
Male left tibiae	62	y=19.450+84.929x	0.7054	0.4977	9.2687
Male right tibiae	63	y=24.982+77.260x	0.7126	0.5078	8.4488
Female left femora	38	y=4.097+104.755x	0.7597	0.5771	9.3665
Female right femora	50	y=1.829+105.431x	0.7976	0.6361	8.4788
Female left tibiae	50	y=22.075+79.674	0.7029	0.4940	9.9859
Female right tibiae	50	y=14.169+90.306x	0.7877	0.6205	8.8445
All left humeri	29	y=22.800+146.978x	0.7867	0.6189	8.5181
All right humeri	31	y=-22.785+146.989x	0.7295	0.5322	9.4605
All left ulnas	31	y=-6.060+122.880x	0.6992	0.4889	10.1675
All right ulnas	31	y=-1.575+118.104x	0.6540	0.4277	10.5699

Key

N - Number of Specimens

MCC - Multiple Correlation Coefficient

CoD - Coefficient of Determination

SEE - Standard Error of Estimate in years

- The mineral content (g/cm^2) - this was measured by ^{125}I photon absorptiometric analysis (Norland-Cameron Bone Mineral Analyser).
- The mineral index (g/cm^2) - this was found by dividing the mineral content (g/cm) by the core length.

90μm thick sections were then removed from each core, ground to a thickness of 80μm and slide mounted with synthetic resin. Four adjacent periosteal fields per section were analysed at x100 with a phase contrast microscope, and measurements were made of:

1) The percentage aggregate osteon lamellar area.
2) The percentage aggregate Haversian canal area.
3) The percentage osteon area.
4) The total number of secondary osteons.
5) The total number of Haversian canals (including primary osteons).
6) The percentage individual osteon lamellar area.
7) The percentage individual Haversian canal area.
8) The aggregate osteon perimeter.
9) The aggregate Haversian canal perimeter.
10) The individual osteon perimeter.
11) The individual Haversian canal perimeter.

From these measurements three ratios were devised:

Ratio 1. Total Haversian area / total secondary osteon lamellae area.

Ratio 2. Total Haversian canal perimeter / total secondary osteon perimeter.

Ratio 3. Individual Haversian canal perimeter / individual secondary osteon perimeter.

All counts and ratios were regressed (stepwise linear regression) against known age, for the men and women together and separately. The results showed that count 3, osteon area, could be used to estimate age at death with the greatest accuracy. The standard error of the estimated ages based on assessments of osteon area ranged from ±7.07 years for the male right femur to ±10.57 years for the left ulna (Table 3.8). The derived regression equations were used to estimate the ages of an additional eight individuals with a known mean age of 40.5 years. Histological assessment yielded a mean estimated age of 41.5 years and the greatest discrepancy between known and estimated age (+5 years) was shown by an eighty year old woman.

3.1.5 SAMSON & BRANIGAN (1987)

Samson & Branigan (1987) observed a great variation in the microstructural preservation exhibited by bodies recovered from Iron Age and Saxon sites in Britain, but noted that Haversian canals could be clearly seen in all specimens. They therefore developed a new method of histological age determination based solely on the quantification of Haversian canals, and it was anticipated that this new technique would be of great value to those studying the most poorly preserved remains. Midshaft lengths of femoral bone were removed from the cadavers of thirty-one men and twenty-seven women ranging in age at death from sixteen to ninety-one years. The femur was selected for analysis as it is amongst those bones most likely to survive in archaeological contexts (Waldron, 1987). The mean cortical thickness of each shaft was recorded, and then two $1cm^2$ pieces were removed from its anterolateral and anteromedial areas, in the mid-third of the cortex (Samson & Branigan, 1987, work as cited in Aiello & Molleson, 1993). Each piece was hand-ground to a thickness of 150µm and slide mounted. Three histological measurements or counts for each bone section were recorded:

1) The mean minimum Haversian canal diameter of thirty canals (as examined at a magnification of x250).
2) The number of Haversian canals per unit area of bone (as counted in back-projected 35mm slides of each section).
3) Haversian canal density (derived as the product of measurements 1 and 2).

The mean cortical thickness and the values of all three histological measurements for each section were regressed against and correlated with known age for both sexes separately. The results showed that:

- The number of Haversian canals per unit area of bone for the male sample showed a strong correlation with age (this value was not disclosed).
- Counts of Haversian canal number in men allowed ages to be determined within ± 8 years.
- The density of Haversian canals in men produced a strong correlation with age and allowed age at death to within ± 6 years.
- Women could only be aged to within ±16 years.

Replicability of the recording methods was tested on a separate 9cm mid-shaft length of femoral bone. Nine sections were made at 1cm intervals along the femoral shaft. Each section was reduced to two 150μm thick ground sections and measurements were recorded as for the original bone sections. Very little variation in histological parameters were noted between the nine sections, and each provided consistent age at death predictions.

3.1.6 ERICKSEN (1991)

Ericksen (1991) devised an additional method of histology-based ageing using bone sections removed from three hundred and twenty-eight known sex and age individuals, from two modern cemeteries in the Dominican republic and from autopsies performed in a Chilean hospital. Undecalcifiedthin sections 1cm thick were removed from all available anterior femoral midshafts, and five fields of view (each 0.886 mm^2) along the periosteal edge of each section were photographed at a magnification of approximately x32.5, using an Olympus camera-mounted microscope and Olympus PM-CBA control unit, for analysis. In each field, counts per mm^2 were made of:

1) X_1 - The number of secondary osteons.
2) X_2 - The number of type II osteons (below).
3) X_3 - The number of fragmentary osteons.
4) X_4 - The number of resorption spaces (below).
5) X_5 - The number of non-Haversian canals.

Type II osteons were described as resulting from "an episode of resorption along a limited stretch of the Haversian canal of a mature osteon .... The new resorption space is relatively small and is

Table 3.9: Results Of Linear Regression Analysis Of The Relationships Between Age And Various Histological Counts As Calculated by Ericksen (1991)

Count	Group	Intercept	Slope	Correlation
Osteons/mm_2	Sexes combined	4.91	0.11	0.49
	Females	6.29	0.09	0.40
	Males	3.26	0.14	0.58
Type II osteons/mm_2	Sexes combined	-0.94	0.04	0.55
	Females	-0.81	0.04	0.59
	Males	-1.05	0.04	0.52
osteon fragments/mm_2	Sexes combined	-16.19	0.60	0.71
	Females	-16.82	0.66	0.73
	Males	-12.77	0.49	0.69
Resorption spaces/mm_2	Sexes combined	2.66	0.01	0.17
	Females	2.47	0.02	0.21
	Males	2.82	0.01	0.13
Non-Haversian canals/mm_2	Sexes combined	7.56	-0.10	-0.66
	Females	7.41	-0.10	-0.69
	Males	7.73	-0.10	-0.63
Average % unremodelled bone	Sexes combined	84.44	-0.96	-0.72
	Females	85.68	-0.99	-0.78
	Males	82.94	-0.94	-0.66
Average % osteonal bone	Sexes combined	25.90	0.28	0.36
	Females	25.87	0.25	0.34
	Males	24.19	0.34	0.41
Average % fragmental bone	Sexes combined	-10.09	0.64	0.69
	Females	-10.56	0.67	0.73
	Males	-7.98	0.57	0.63

eventually provided with a cement line and filled in with new lamellae, forming an osteon-within-an-osteon structure" (Ericksen; 1991, p. 174). Resorption spaces were described as "distinguished by their scalloped edges formed by Howship's lacunae and range from the first tiny breakthrough of its walls by a vessel in unremodelled lamellar bone or within the Haversian canal of an osteon to the grossly visible resorption cavities in the bones of older individuals" (Ericksen; 1991, p. 174).

In addition to the above counts, "a 100-space grid was superimposed upon the photographs and each space counted according to the predominant microstructure type, adding three further variables to the study" (Ericksen; 1991, p. 175):

1) X_6 - Unremodelled bone.
2) X_7 - Osteonal bone.
3) X_8 - Fragmentary bone.

To determine their relation to age and to produce regression equations that could be used for age determination, each of the eight histological counts was subjected to stepwise regression analysis for the males, females and sexes combined (Table 3.9). Statistical analysis showed that:

- All counts, except the number of resorption spaces per mm^2, showed a highly statistically significant correlation with age.
- As expected, non-Haversian canal number per mm^2 and percentage unremodelled bone were negatively correlated with age, and all other histological features were positively correlated with age.
- Most derived regression equations had standard errors of estimate of approximately ± 10 years.
- Sex-specific regression equations gave better results than non-specific equations.

It was noted that some of the regression equations, especially those relying on just one or two histological counts, were "unrealistic as age predictors". For example the regression equation based on the male fragmentary osteon count was found to be useless for those aged under forty-four years. In practice, Ericksen identified three equations (Table 3.10) as giving the most satisfactory results:

Table 3.10: Best Combinations Of Histological Counts (x) For Estimating Age (y) As Identified By Ericksen (1991)

Group	Regression Equation	r^2	SEE
Sexes combined (N=328)	$y=67.43+1.11x_1+2.46x_2+0.20x_3-1.57x_5-0.30x_6-0.39x_7$	0.67	±10.08
Females (N=154)	$y=63.39+0.55x_1+3.12x_2+0.20x_3+0.92x_4-1.57x_5-0.31x_6-0.24x_7$	0.71	±10.00
Males (N=174)	$y=57.98+1.36x_1+1.90x_2+0.32x_3-1.62x_5-0.17x_6-0.33x_7$	0.64	±10.05

<u>Key</u>

x_1: The number of secondary osteons.

x_2: The number of type II osteons.

x_3: The number of fragmentary osteons.

x_4: The number of resorption spaces.

x_5: The number of non-Haversian canals.

x_6: Unremodelled bone.

x_7: Osteonal bone.

x_8: Fragmentary bone.

r^2: Correlation coefficient squared.

SEE: Standard error of the estimate.

3.1.7 Stout & Paine (1992)

Stout & Paine (1992) prepared bone sections for histological analysis from the middle third of the left sixth rib and from the mid-shaft of the left clavicle from forty individuals of known age (including seven females and one sex unknown). For each section, measurements were made of:

1) The cortical area - defined as "the sum of the areas of cortical bone contained within all of the microscopic fields examined per bone" (Stout & Paine; 1992, p. 112).
2) Intact osteon density - defined as the number of secondary osteons which had at least 90% of their Haversian canal perimeter untouched by remodelling.
3) Fragmentary osteon density - this included a count of those osteons not recorded in 2.
4) Total visible osteon density - the total of counts 2 and 3.

Age-predicting models for the rib and clavicle, separately and combined, were calculated using regression analysis, with total visible osteon density as the independent variable and log age as the dependent variable (Table 3.11):

Table 3.11: Age Predicting Regression Equations Devised By Stout & Paine (1992)

Bone	**Regression Equation**	**r**
Rib	y=2.343+0.050877xr	0.849
Clavicle	y=2.216+0.070280xc	0.836
Rib and Clavicle	y=2.195+0.029904xr+0.035430x c	0.881

<u>Key</u>

xr=total visible osteon density in the rib.

xc=total visible osteon density in the clavicle.

y=log age.

Differences between actual and predicted age were lowest for the rib (they ranged from -2.7 to 9 years) and highest for the clavicle (they ranged from -2.5 to 14.5). Stout & Paine described their method as particularly useful because it showed that bones other than the long bone and the mandible could be used for histology-based age determination.

3.1.8 WALLIN *et al.* (1994)

Wallin *et al.* used a digital image analysis system to aid quantification of histological features. Initially, undecalcified 80 μm thick cross-sections were prepared from the left femurs of fifty-two men and forty-nine women, ranging in age at death from eighteen to eighty-seven years. The medical history of each individual was known, and the sample included individuals who had suffered from endocrine disease, malignant neoplasms and some who had been treated by long-term intake of synthetic hormones, however in no case was gross or microscopic pathology associated with the left femur itself. Sections were stained with toluidine blue and were examined in a photomicroscope.

Four fields of view, as recommended by Ahlqvist & Damsten (1969), were photographed and an additional photograph of the most anterior part of the bone section was also taken. Prints were produced at a magnification of x100.47, and each showed an area of bone which measured 2.15 by 1.45 mm.

A ball pen stylus, connected to a MINI-MOP digital image analysis system, was used to trace the outline of each secondary osteon and the outline of its central Haversian canal in each photograph. Secondary osteons were defined as 'concentric structures with a number of concentric lamellae around a central intact (Haversian canal) with a clearly defined border (reversal line) with other osteons and/or lamellar bone'. All secondary osteons including those partially resorbed were traced, and those on the edge of the photographic field were also traced providing their Haversian canal was entirely within the photographic field. These tracings allowed four histological measurements and counts to be recorded, although they were not clearly defined:

1. The osteon area.
2. The minimum diameter of osteon.
3. Osteon count.
4. The total perimeter.

Measurements were averaged for each individual, and analysed with respect to known age, sex and to the presence of chronic disease/long-term intake of medication prior to death. This analysis showed that:

- Osteon area, osteon count and total perimeter increased more slowly with advancing age, and the presence of chronic disease/long-term intake of medication prior to death was found further to delay this process. This latterpoint opposes the findings of Kerley (1965), who did not recognise any disruption of normal turnover in bones from individuals who had suffered documented chronic disease (above).
- Osteon area, osteon count and total perimeter had lower values for males than females.
- Osteon number, for both sexes, showed the 'best' correlation with age, although no coefficient was presented.

- Total perimeter showed the 'second best' correlation with age, again no coefficient was presented.
- Osteon areas 'were shown to correlate badly' with age, again no coefficient was presented.

Four regression equations for predicting age, each based on the average values of one of the four histological measurements, with codes representing sex and medical history were fitted to the data. In this way, four separate histology-based ages were calculated for each individual, and a further equation was fitted to the resulting data. This final equation allowed the four separate histology-based ages for each individual to be converted into a single age at death estimate, which had a standard deviation from true age of 'a little less than ± 13 years'.

3.1.9 In Summary

- Bone histology-based ageing methods have been used to determine age at death in modern human populations (Kerley, 1965; Kerley & Ubelaker, 1978; Ahlqvist & Damsten, 1969; Singh & Gunberg, 1970; Thompson, 1979; Samson & Branigan, 1987; Ericksen, 1991; Stout & Paine, 1992; Wallin *et al.*, 1994), in archaeologically recovered human remains (Kerley, 1965; Samson & Branigan) and in a modern laboratory rat population (Singh & Gunberg 1971).
- These methods have been developed on the quantification of histological features in limb bones (Kerley, 1965; Kerley & Ubelaker, 1978; Ahlqvist & Damsten, 1969; Singh & Gunberg, 1970, 1971; Thompson, 1979; Samson & Branigan, 1987; Ericksen, 1991; Wallin *et al.*, 1994), the mandible (Singh & Gunberg, 1970; 1971), the clavicle and the rib (Stout & Paine, 1992).
- Although not used to determine age, histological features (whose development is age related) have been noted in the bones of a variety of other mammalian orders, including artiodactyla (Enlow & Brown 1958; Enlow, 1968).

It should be noted that there is an element of difficulty in assessing the results of the above bone histology-based age determination studies, as they do not give details of tests carried out on the fit of the regression models used. Most studies, but not Wallin *et al.* (1994), present age as the dependent variable (y) and histological count as the independent variable (x). This makes it easier to express the accuracy of the resulting age prediction equation as a standard error of estimate (Table 3.12), but it is not a model that makes sense in biological terms - age is not a function of, or dependent on bone histology, but rather bone histology is a function of, or is dependent on age. Therefore, it is more appropriate to fit a regression model in which age is the independent variable (x) and histological count is the dependent variable (y). This is an approach that was used by Wallin *et al.* (1994). By applying reverse regression procedures (that is turning the regression equation around), ages can still be predicted and the accuracy of the estimate can be more appropriately be expressed through measures of bias and inaccuracy (Lovejoy *et al.*, 1985; Chapter 5.4).

Table 3.12: Least Standard Error Of Age Estimate Expressed By Those Who Regressed Age (y) On Histological Count (x)

Author	**Least Standard Error Of Age Estimate**
Kerley (1965)	± 5.27 years
Ahlqvist & Damsten (1969)	± 6.71 years
Singh & Gunberg (1970)	± 2.55 years
Singh & Gunberg (1971)	± 0.117 log days
Thompson (1979)	± 7.07 years
Samson & Branigan (1987)	± 6 years
Ericksen (1991)	± 10.08 years
Stout & Paine (1992)	not reported

3.2 Comparisons Of Microscopic And Macroscopic Age Determination Techniques

The effectiveness of bone histology-based ageing in human material has been compared directly against routine macroscopic age determination techniques in four studies:

3.2.1 Pfeiffer (1980)

3.2.2 Thompson & Trinkaus (1981) and Trinkaus & Thompson (1987)

3.2.3 Aiello & Molleson (1993)

Several macroscopic age estimation techniques used in these studies are almost exclusively applied to humans and, consequently, were not discussed in Chapter 1, when consideration was given to age estimation in deer. These techniques are reviewed briefly:

- **The Pubic Symphysis:** The right and left pubic bones are joined anteriorly in the midline, by symphyseal cartilage, to form the pubic symphysis. Each pubic bone has a symphyseal surface or face, the appearance of which changes gradually throughout life due to normal growth and remodelling processes (Chapter 2), and standards have been detailed for the state of development observed in modern populations with respect to age (Todd, 1920; 1921; McKern & Stewart, 1957; Gilbert & McKern, 1973; Brooks, 1955; Ascádi & Nemeskéri, 1970).
- **Auricular Morphology:** The auricular surface of the posterior ilium also shows age related morphological changes. These may be assessed through gross observations of the bone surface and can be compared with standards developed for known age human populations (Kobayashi, 1967; Lovejoy *et al.*, 1985).
- **Cranial Suture Obliteration:** The skull or cranium is made up of separate flat bones, which meet at joints called sutures. Bony bridges gradually develop between the bones and, with increasing age, the sutures tend to be obliterated. The degree of closure and/or obliteration may be compared to standards developed from studies of modern humans (Todd & Lyon, 1924; 1925; Brooks, 1955; Krogman, 1962).

3.2.1 PFEIFFER (1980)

Pfeiffer (1980) aged six skeletons from a Late Archaic burial in Ontario using the Ahlqvist & Damsten (1969) technique (above), and compared the resulting ages with those obtained through gross examination of the pubic symphysis (Todd, 1920; McKern & Stewart, 1957; Gilbert & McKern, 1973) and through assessments of cranial suture obliteration (Krogman, 1962) (Table 3.13).

Table 3.13: A Comparison Of Ages Determined By Pfeiffer (1980) Using Microscopic And Macroscopic Techniques

Burial	Sex	Ahlqvist & Damsten Ages (in years)	Pubic Symphysis Ages (in years)	Cranial Suture Ages (in years)
3	F	43.3 (± 6.71)	29.2a (23 to 39) 36.9b (30 to 47)	no fusion<30
9	F	27.7 (± 6.71)	29.2a (23 to 29) 33.0b (22 to 40)	no fusion<30
10	M	48.1 (± 6.71)	41.0a (36+)	late 40s or late 50s
19	M	38.6 (± 6.71)	35.8a(29+)	late 40s
20	M	24.3 (± 6.71)	24.1a (22 to 28)	late 20s or late 30s
22	M	21.8 (± 6.71)	22.4a (20 to 24)	no fusion< 30
Mean Ages		34.0 (± 6.71)	31.3a, 32.2b	-

<u>Key</u>

a - McKern & Stewart (1957) norms

b - Gilbert & McKern (1973) norms

Five specimens showed good matches between the various determination techniques, although cranial-suture ages did not always match with other estimates. Closest agreement was between the Ahlqvist & Damsten ages and the pubic symphysis ages. Although the accuracy of each age determination technique could not be assessed (as the true ages of the specimens were unknown) a general level of agreement between them was found and it was concluded that "one can confidently substitute microstructural age estimates for the more traditional pubic-symphysis age estimates" (Pfeiffer; 1980, p. 793). However, due to the time and expense involved in producing bone sections for microstructural analysis the Ahlqvist & Damsten technique was recommended only in situations when the pubic symphysis is not available.

3.2.2 Thompson & Trinkaus (1981) And Trinkaus & Thompson (1987)

Thompson & Trinkaus found close agreement between macroscopic and microscopic estimates of age at death for the Shanidar 3 Neandertal, recovered from the upper Mousterian levels of Shanidar Cave in northern Iraq, dating to at least 45 000- 50 000 BP. Macroscopic age at death estimates were based on observations of the pubic symphysis (McKern & Stewart, 1957) and of the auricular surface (Kobayashi, 1967). The pubic symphysis-based estimate was 'at least 38 years', and the auricular surface-based estimate was 'at least 40 years, probably between 40 and 50 years'. These estimates were seen to be supported by the presence of advanced osteophyte formation on the lower thoracic and upper lumbar vertebrae, and development of exostoses. The teeth showed extensive occlusal attrition and suggested a more advanced age than 40 years, but it was noted that attrition may have occurred at a faster rate in Neandertals (Hillson, 1986). It was concluded that the macroscopic age determination techniques suggested an age of death of at least 40 years.

The microscopic age at death estimation was based on femoral osteon and Haversian canal area (Thompson, 1979). The Shanidar 3 femoral fragment was very fragile and had to be embedded in epoxy prior to sectioning but, once embedded, an 80μm thin section was cut, slide mounted and examined with a phase-contrast microscope. Despite extensive post-mortem replacement of the organic and mineral phase of the bone ('diagenesis' - Chapter 4), secondary osteons could clearly be seen and quantified in the prepared section. The microscopic age estimation technique yielded an age at death of 42 $\pm$ 8.2 years, and this agreed well with the macroscopic-based age estimations, thus increasing confidence that they had provided a reasonably accurate age at death estimate. It was concluded that histology-based age estimation was a reliable method for ageing fossil hominids, and that it was extremely useful when human remains were too badly fragmented to permit macroscopic-based age estimates.

In a later study, Trinkaus & Thompson (1987) re-compared macroscopic and microscopic age estimates at death for the Shanidar 3 Neandertal, and compared similar estimates for Shanidar 4, 5 and 6. Like Shanidar 3 (above), Shanidar 5 was recovered from the upper Mousterian levels of the

Shanidar Cave and dated to at least 45 000 BP. Shanidar 4 and 6 were recovered from a multiple burial in the middle Mousterian levels, which dated to at least 60 000 BP (Trinkaus & Thompson, 1987). As all four skeletons were incomplete, a mixture of macroscopic ageing techniques had to be employed (Table 3.14), and these included observations of dental attrition, the pubic-symphysis, auricular morphology, suture closure and non-traumatic degenerative changes (Trinkaus, 1983). According to macroscopic age estimates Shanidar 6 was the youngest of the four specimens, at around 25 years, Shanidar 3 was again placed at around 35 to 45 years, and Shanidar 4 and 5 had similar age estimates of between 35 and 50 years.

Table 3.14: Trinkaus & Thompson's (1987) Age Estimates For The Shanidar 3, 4, 5 And 6 Neandertals

Skeleton	Macroscopic Technique Employed	Macroscopic Age Estimate (years)	Microscopic Age Estimate (years)
Shanidar 3	Dental Attrition, Auricular Morphology, Pubic Symphysis, Non-traumatic Degenerative Changes	30-45	41 ±6.7
Shanidar 4	Dental Attrition, Suture Closure, Non-traumatic Degenerative Changes	35-50	36 ±6.7
Shanidar 5	Dental Attrition, Suture Closure, Non-traumatic Degenerative Changes	35-50	40 ±6.7
Shanidar 6	Dental Attrition, Non-traumatic Degenerative Changes	20-35 (probably 25)	24 ±6.7

Microscopic age estimates were based on the quantification of femoral osteon area and osteon number (Thompson, 1979). All four Neandertals had surviving femoral fragments, but these were extremely fragile and exhibited post-mortem cracks as well as microscopically observed 'post-mortem replacement of bone mineral with other minerals'. Nevertheless, generally excellent preservation of age-related histological detail was noted. Thin sections for the Shanidar 4, 5 and 6 Neandertals were prepared in similar manner to that of Shanidar 3 (above) and, for each section, secondary osteon number and secondary osteon area per mm^2 were recorded with the aid of a semiautomatic image analyser (Thompson & Galvin, 1983, cited in Trinkaus & Thompson, 1987). Secondary osteon area (X) was calculated for each section and inserted into the regression equation:

Age=101.9(X)+6.677. This regression equation was modified from Thompson (1979), and was slightly different to that used in the previous study to age the Shanidar 3 Neandertal and so resulted in a slightly different age estimate (Table 3.14). Generally, good comparisons between ages obtained through macroscopic and microscopic techniques were noted. Again, although the absolute accuracy of all the selected age estimation techniques was unknown, their compatibility did increase the confidence that they had provided reasonably accurate age at death estimates. It was further concluded that such agreement between the various age estimates suggested that development rates in the Shanidar Neandertals were similar to those in anatomically modern humans.

3.2.3 AIELLO & MOLLESON (1993)

Aiello & Molleson tested the accuracy of two histology-based and three macroscopic age determination techniques on ten men and ten females of known age at death from Christ Church, Spitalfields, London (18th and 19th century).

3.2.3.1 Microscopic Techniques

Histology-based age determination techniques were adopted from the works of:

- Kerley (1965)/Kerley & Ubelaker (1978)
- Samson & Branigan (1987)

- **Kerley (1965)/Kerley & Ubelaker (1978)**

Two slight modifications were made to the Kerley/Kerley & Ubelaker technique:

1. The microscopic field locations recommended by Ahlqvist & Damsten (1969) were used, instead of those recommended by Kerley.
2. The Kerley (1965) 80% complete definition for osteons was found to be unworkable. Instead, osteons were defined as complete if their central canal was untouched by remodelling, and as fragments if their central canal was encroached by remodelling.

Table 3.15: Correlation Of Kerley (1965) Based Counts And Known Age In The Spitalfields Population (after Aiello & Molleson, 1993)

Bone Histology Count	Total	Females	Males
	(N=20)	(N=10)	(N=10)
% lamellar bone	-0.81*	-0.82†	-0.81†
secondary osteon number	0.80*	0.71‡	0.92*
fragmentary osteons	0.82*	0.86†	0.79†
primary osteon number	-0.81*	-0.79†	-0.82†

Key

* - $P<0.001$

† - $P<0.01$

‡ - $P<0.05$

Table 3.16: Correlation Of Kerley (1965)/Kerley & Ubelaker (1978) Based Ages And Known Age In The Spitalfields Population, n=20, (after Aiello & Molleson, 1993)

Age Estimate	Correlation
% lamellar bone age	0.82*
Secondary osteon age	0.76*
Fragmentary osteon age	0.77*
Primary osteon age	0.78*
Average histological age	0.88*

* - $P<0.001$

Table 3.17: Statistical Analysis Of Kerley (1965)/Kerley & Ubelaker (1978) Based Ages For The Spitalfields Sample, n=20, mean known age=54.7, known age range 15-91 years, (after Aiello & Molleson, 1993)

Calculation	**K1**	**K2**	**K3**	**K4**	**K5**
Mean inferred age	59.9	38.0	50.7	38.5	46.8
Deviation of mean inferred from mean known age	+5.22	-16.7	-4.0	-16.1	-7.9
Average difference between inferred and known age	19.0	20.4	12.5	17.2	10.1
Standard error of the estimate	17.4	9.8	8.3	11.5	8.9
Percentage of individuals with inferred ages:					
within ± 5 years of known age	25%	5%	20%	15%	30%
within ± 5 to 10 years of known age	1%	4%	5%	5%	7%
more than 10 years of known age	14%	15%	11%	12%	7%

Key

K1 - Kerley/Kerley & Ubelaker secondary osteon age

K2 - Kerley/Kerley & Ubelaker fragmentary osteon age

K3 - Kerley/Kerley & Ubelaker percentage lamellar age

K4 - Kerley/Kerley & Ubelaker primary osteon age

K5 - Average of K1-K4

Partial cross sections of bone, 0.5 cm thick, were removed from the anterior midshaft of the left femur of each individual. Each section was then "mounted on slides proximal side up, fixed in Epotek 301 two-part epoxy resin under pressure in spring-loaded presses, and cold cured overnight. They were then trimmed to a thickness of 500 μm on a trimsaw and ground down to 50 μm using a Jones and Shipman surface grinder with a cup-wheel diamond grindhead." (Aiello & Molleson; 1993, p. 691). Then, as Kerley, Kerley & Ubelaker, ground cross sections were examined under transmitted light with a Nikon Optiphot polarising microscope at a magnification of x100. Four fields of view per section, located as recommended by Ahlqvist & Damsten (1969), were examined and each had a diameter of 1.77mm at the level of the bone section. For each specimen the total numbers of secondary osteons, fragmentary osteons and primary osteons in all four fields were determined. The percentage area of circumferential lamellar bone was also recorded in each microscopic field, using a graticule. The graticule was orientated so that its grid rows ran parallel to the circumferential lamellae, and the number of rows filled with circumferential lamellar bone were then simply counted and averaged to produce a single figure for each section. Aiello & Molleson stated that, for compatibility, all counts 'were then scaled to the field of view used in Kerley & Ubelaker's analysis'. There were strong significant correlations between the counts and known ages (Table 3.15), and strong correlations (significance level not stated) between estimated ages from Kerley & Ubelaker's equations and known ages (Table 3.16). However, the estimated ages were found to have much larger deviations from known age than indicated by Kerley & Ubelaker (Table 3.17) and, although these were partially attributed to modifications made in this study (field location and definitions of complete and fragmentary osteons), two other causal factors were identified:

1) The use of third-order regression equations for some of the best-fit lines. The third-order regression equation fitted to the relationship between age and fragmentary osteon number did not indicate a consistently progressive increase in fragmentary osteon number with increasing age, as is the case in reality but instead suggested that fragment number peaked at 80 years and then subsequently reduces with increasing age. This resulted in under-predictions of age in those older specimens with an average > 90 fragmentary osteons per field of view. Similarly, the third-order regression for the relationship between age and primary osteon number was "biologically

difficult to explain" for those < 20 years old, and it produced a maximum age of 58 years. Again this resulted in serious under-estimation of age for the older specimens.

2) Specimens in the Spitalfields sample had a different bone remodelling rate from those in Kerley's sample. In the Spitalfields sample young bone (represented by circumferential lamellae and primary osteons) was retained to a much older age than in Kerley's sample. Consequently, the Kerley & Ubelaker best-fit lines for percentage of circumferential lamellae and primary osteons seriously under-predict the ages of the young specimens in the Spitalfields sample.

However, the Kerley & Ubelaker regression equations did produce "reasonable estimates for individual age" when the results of all the predictive equations were taken together to produce an average histological age (AHA). Thirty percent of Spitalfields specimens had an AHA within ± 5 years of true age, and a further thirty-five percent had an AHA within ± 5 to 10 years of true age (Table 3.17). It was concluded that the Kerley, Kerley & Ubelaker technique produced ages to within 10 years for two thirds of the sample, and that the technique was "fairly robust ... in that it achieved this accuracy in spite of line-fitting peculiarities and modifications in relation to the definition of the osteon fragments and to the position of the fields of view ..." (Aiello & Molleson 1993, 695).

- **Samson & Branigan (1987)**

Aiello & Molleson were able to apply the Samson & Branigan ageing technique to 78 individuals within the Spitalfields population. This was because this technique was based on the quantification of histological features in two fields in the mid-cortex of the femur, and more individuals in the Spitalfields population had intact mid-cortical femoral bone. Two histological features were recorded:

1. The total number of secondary osteons - Bone fields were examined with a Nikon microscope at a magnification of x100. A square graticule was used to delimit an area of bone measuring $0.96mm^2$ at the section level.

2. The average minimum osteon diameter - This was determined on the basis of the measurement of 30 randomly selected intact secondary osteons in each field. Measurements were made using the Nikon microscope at a magnification of x400.

The two measurements were then multiplied together to obtain secondary osteon density (above). Analysis of the resulting data revealed that the Samson & Branigan technique 'did less well' than the Kerley/Kerley & Ubelaker technique:

- Generally, it yielded lower correlations between known age and histological counts than for the Kerley/Kerley & Ubelaker technique (Table 3.18). Only the measurements of secondary osteon diameter for both men and women, and secondary osteon density for men, showed strong correlations with known age.
- When the measurements of secondary osteon diameter (both men and women) and secondary osteon density (men only) were subjected to regression analysis and used to determine age resulting predictions were accurate only to within ± 19.2 years. This compared markedly with the standard error of ± 6 years for Haversian canal density in men reported by Samson & Branigan.

Table 3.18: Correlation Of Samson & Branigan (1987) Based Counts And Known Age In The Spitalfields Population (After Aiello & Molleson, 1993)

Bone Histology Count	Total	Females	Males
	(N=74)	(N=24)	(N=50)
osteon number	0.05*	-0.21*	0.20*
average osteon diameter	0.24*	0.33*	0.20*
secondary osteon density	0.19*	-0.001*	0.30‡

Key: *not significant ‡$P<0.05$

The failings of the Samson & Branigan technique were attributed to their recommendation of the use of inner mid-third areas of the femoral cortex and not the peripheral areas as in Kerley (1965). Aiello and Molleson noted Pfeiffer's (1992) comment that the study of osteon remodelling in the mid-cortex could result in gross over estimation of ages, and they agreed that "the outer periphery of the cortex (sampled by the Kerley technique) may respond to age changes in a more consistent fashion than do the deeper layers" (Aiello & Molleson 1993, 696). The failings should be viewed with caution as the features recorded by Aiello & Molleson were not necessarily the same as those recommended by Samson & Branigan (1987):

- Samson & Branigan recorded 'the number of Haversian canals' and 'the mean (minimum) Haversian canal diameter'.
- Aiello & Molleson recorded 'the number of secondary osteons' and 'the mean minimum secondary osteon diameter'.

Neither Samson & Branigan nor Aiello & Molleson defined the histological terms they used. It is likely that Aiello & Molleson produced smaller counts of secondary osteons than Samson & Branigan of Haversian canals, because Aiello & Molleson only counted secondary osteons, whereas Samson & Branigan counted all those Haversian canals that formed parts of primary osteons, secondary osteons and fragmentary osteons. Aiello & Molleson also produced larger measurements of mean minimum secondary osteon diameter than Samson & Branigan did of Haversian canals, because the diameter of a complete secondary osteon extends far beyond that of its central Haversian canal. Currently, there is no evidence to suggest that secondary osteon diameter alters in respect to age, although Jowsey (1960) did note that Haversian canals appeared to be larger in older individuals than in younger individuals.

Although Aiello & Molleson may not have truly replicated the Samson & Branigan technique, they did carry out further investigations of the structure of the mid-cortex which confirmed "that histological features in the middle of the cortex do not reflect age to the same degree as they might at the periphery of the cortex" (Aiello & Molleson 1993, 697). These further investigations involved using an image analyser to record "the total aggregate area occupied by secondary osteon lamellae and osteon canals (including primary osteons) in three fields in the centre of the cortex located medially, anteriorly and laterally" (Aiello & Molleson 1993, 696) in each of fifty-one femoral cross sections (twenty-five men and twenty-six women). Aggregate osteon area counts were correlated with known ages the sexes together and separately, and were entered into Thompson's (1979) aggregate osteon area regression equations for predicting age, and were used to re-estimate age for the selected fifty-one Spitalfield individuals. "A significant, but low correlation for the women was noted (Table 3.19), with a relatively large standard error of estimate - 18.2 years" (Aiello & Molleson; 1993, 696).

Table 3.19: Correlation Of Aggregate Osteon Area Counts In The Central Cortex And Age As Calculated By Aiello & Molleson (1993)

	Whole Sample (N=51)	Men (N=25)	Women (N=26)
Aggregate Osteon Area	0.24*	0.002*	0.40‡

<u>Key</u>
*not significant
‡$P<0.05$

It was noted that Thompson (1979) had found aggregate osteon area in the outer cortex to be a useful measure for predicting age, and so it was again decided that the field location and not the feature count was at fault here. Thus the Kerley/Kerley & Ubelaker technique, which was based on quantifications of histological structures in the outer cortex, was preferred to the Samson & Branigan technique, based on the inner cortex.

3.2.3.2 Macroscopic Techniques

Macroscopic age estimations were based on the pubic-symphysis techniques of:

- McKern & Stewart (1957) and Gilbert & McKern (1973), abbreviated to McKSG.
- Todd (1920, 1921) and Brooks (1955), abbreviated to TB.
- Acsádi & Nemeskéri (1970), abbreviated to AN.

These techniques were selected over other macroscopic age estimation methods as "pubic-symphysis ageing has been considered to give some of the most reliable results" (Aiello & Molleson; 1993, p. 698). However it is noted that, although the Acsádi & Nemeskéri technique was recommended by the Workshop of European Anthropologists (1980), Brooks & Suchey (1990) found difficulty in applying it to their sample of 1012 bodies: 48% of males and 47% of females in the Brooks & Suchey sample did not correspond to any of Acsádi & Nemeskéri five phases of development, but fell

Table 3.20: Various Pubic Symphyis-Based Age (in years) At Death Estimates For The Spitalfields Sample (after Aiello & Molleson, 1993)

	Acsádi & Nemeskéri Estimates (N=64)	Todd/Brooks Estimates (N=64)	McKern, Stewart & Gilbert Estimates (N=65)
Mean Estimated Age	55.6	37.7	33.4
Deviation of mean estimated age from mean known age	+0.9	-17.1	-22.2
Range of Estimated Ages	26.3-68.5	18.5-51.0	19.8-55.7
Average difference between estimated and known age	10.8	18.2	22.5
Standard error of the estimate	7.9	12.5	15.0

Table 3.21: The Accuracy Of The Various Pubic-Symphysis Based Ages (in years) For The Spitalfields Sample (after Aiello & Molleson, 1993)

Percentage of individuals with estimated ages:	Acsádi & Nemeskéri-Based Estimates (N=64)			Todd/Brooks-Based Estimates (N=64)			McKern/Stewart/Gilbert-Based Estimates (N=65)		
	All	<45	>45	All	<45	>45	All	<45	>45
within ± 5 yrs of known age	28.0	17.7	31.9	20.3	52.9	8.5	13.6	31.3	8.2
within ± 5 to 10 yrs of known age	23.4	17.7	25.5	14.1	29.4	8.5	15.2	43.8	6.1
over 10 years of known age	48.4	64.7	42.6	65.6	17.7	83.0	71.2	23.5	85.7

between phases 3 and 4, and those bodies that could be assigned a phase were much younger than Acsádi & Nemeskéri suggested.

Ages obtained through the application of the selected macroscopic techniques were compared with known ages (Table 3.20), and the accuracy of each for ageing those under 45 years and those over 45 years was tested (Table 3.21). Both the TB and McKSG techniques 'seriously under-predicted the ages for individuals known to be over 45 years old' and the AN technique produced ages that were accurate to within ± 10 years for the 40 to 70 year olds, but over-predicted the ages of those under 40 years and those over 70 years. The AN technique also produced a population mean age estimate and individual age estimates that more closely matched the known ages of the Spitalfields sample than either of the other two techniques, and consequently, was considered to have a greater overall reliability. It was noted that the AN technique was developed on a population than had an age distribution that more closely matched the Spitalfields population and so was more likely to produce closer age estimates than either the TB or McKSG techniques.

Subsequent comparisons between microscopic and macroscopic age estimation techniques were confined to assessments of those ages obtained using the Kerley/Kerley & Ubelaker technique and the Acsádi & Nemeskéri technique:

- Known age was highly correlated with histological variables in the periphery of the bone cortex.
- The accuracy claimed for histology-based ageing on the basis of one population does not necessarily apply to individuals from another population.
- Histology-based ages are not necessarily more reliable than ages determined from the appearance of the pubic-symphysis, but neither are they any worse.
- The Acsádi & Nemeskéri macroscopic ages were as accurate as the Kerley, Kerley & Ubelaker microscopic ages, but the Acsádi & Nemeskéri mean population age was more accurate than that of Kerley, Kerley & Ubelaker.

Aiello & Molleson (1993, 702) stated that their conclusions "may not be generally applicable to all samples" and that their findings may simply be a product of the fact that age distribution within the Spitalfields sample is similar to that of the Acsádi & Nemeskéri reference sample. Furthermore they stressed that all age determination techniques reflect the age structure and or conditions of the reference population on which they were developed and, in the case of histology-based ageing, more needs to be known about the factors governing cortical remodelling before more generally applicable age determination equations can be developed. Finally, it was suggested that for the most accurate determinations of individual and sample mean age combinations of the histology-based ageing and pubic-symphysis ageing should be used.

3.2.4 IN SUMMARY

- Generally, good agreement between histology-based age estimates and more traditional macroscopic age estimates were noted.
- Histology-based ageing methods have an advantage over macroscopic age estimation methods in that they can be applied to highly fragmented archaeological remains.

3.3 In Conclusion

Over the last thirty years, a variety of histology-based age determination methods have been developed, but Kerley's (1965) pioneering method still provides the most accurate age estimates (Bouvier & Ubelaker, 1978; Stout & Gehlert, 1980; Stout & Stanley, 1991), and resulting ages are comparable to those obtained by more traditional macroscopic age determination methods (Aiello & Molleson 1993), and evidence suggests that the technique may be of use in the age determination of a variety of non-human mammals (Enlow, 1968; Singh *et al.*, 1974). Consequently, it was decided that those features isolated by Kerley (1965) as age related in humans (secondary and fragmentary osteons, non-haversian canals and circumferential lamellae area) would, similarly, be quantified in roe deer, and their value as an age indicator in that species

CHAPTER 4 MATERIAL AND METHODS

4.1 The Sample Material

In order to establish a system of age determination based on the quantification of microscopic bone changes in a non-human mammalian population, 189 roe deer mandibles were considered. Roe deer mandibles were selected for analysis as:

- The lower part of the mandibular ramus consists of cortical bone, and is comprised of those histological features (non-Haversian canals, secondary osteons, fragmentary osteons and circumferential lamellae) which in man have been noted to show age-related patterns of development (Chapter 3).
- A large sample was available.
- The sample was believed to be of known age.
- The sample contained individuals from each year of life, from birth through to 14 years.
- It was hoped that the pattern of age-related histological development isolated in a modern 'wild' population would be representative of that experienced by similar ancient stock, as opposed to growth rates in modern highly bred domestic stock which are known to be different from comparative primitive breeds (Chapter 1).
- Roe deer are the most ubiquitous European deer and they are prevalent in the archaeological record. For example, 103 roe deer bone fragments, including 17 right mandibles, were recovered from the important early Mesolithic site of Star Carr, in the Vale of Pickering, Yorkshire. In their re-analysis of the fauna at Star Carr, Legge & Rowley-Conwy (1985) used the roe deer remains to infer that the site was occupied in the summer months and not winter as previously thought (Clark, 1954). They noted that 14 roe deer jaws from Star Carr had either heavily worn deciduous teeth or the permanent premolars in the early stages of eruption. Modern roe deer are all born around the beginning of June (Ratcliffe & Mayle, 1992). The youngest Star Carr jaw corresponded to that of modern animals killed in their first spring and the other young Star Carr jaws indicated a very restricted kill period of just a few months, over the earlier part of the summer, whereas none showed the permanent premolars or the third molar in the later stages of eruption - a growth stage in modern roe deer known to occur in their second winter. All other

jaws at Star Carr exhibited tooth wear indicative of animals aged over two years but unfortunately, as Davis (1987, p. 77) reported, 'after the second year (in roe deer) variability in tooth wear is too great to allow accurate seasonal dating from dentition'.

The mandibles used in this study were very kindly donated by the Forestry Commission, Alice Holt Lodge, Wrecclesham, Surrey. The sample material consisted of 68 buck and 121 doe mandibles, and was made the main focus of the project on the understanding that most animals were tagged at birth so that their exact age at death was known. It transpired much later that the animals were instead aged through a cement annulation technique (Ratcliffe & Mayle, 1992; Chapter 2) which has been routinely adopted by the Forestry Commission and is widely used throughout the world for wildlife management. A total wear score for the molars in each mandible was kindly supplied by Richard Carter (Chapter 1), at that time an undergraduate student at the Institute of Archaeology, who was developing a dental development scoring scheme for roe deer, based on the standards developed by Brown & Chapman (1991a; 1991c; Chapter 2) for fallow deer and red deer, and required access to a large sample of known age roe deer material. He was granted access to the mandibles used in this study and the x-rays that were taken of them after they had been cleaned (below) and in return made available the attrition scores.

The mandibles had been removed from animals ranging in age from kids through to one individual in its fourteenth year of life, and which had been culled within legal limits over a 3 year period in Craigellachie, Scotland. Culling is integral to land and herd management. It has been suggested that 30% of a herd living within a bounded area should be culled each year, to avoid intense competition for food and prolonged death by starvation (Chapman & Chapman, 1975). The roe deer buck culling season runs from 1 April to 20 October, and the doe season runs from 21 October to 31 March. At Craigellachie, most doe culling is carried out within the early part of the season, when kids are targeted before their mothers to avoid orphaning. Similarly most buck culling occurs early in their season, when movement of younger animals is greatest. Consequently, kids and those under 6 years form the easiest targets and greater proportions of annual quotas (pers. Comm. Malcolm Hobson, Forestry Commission, Craigellachie), over 75% of the specimens supplied were aged under

Table 4.1: The Donated Craigellachie Roe Deer Sample. Ages are cement layer ages, and are given in years and months as provided by the Forestry Commission.

Bucks 1983 cull	
Specimen	**Age**
CGC1/83	3.11
CGC2/83	6.0
CGC3/83	3.0
CGC5/83	2.0
CGC6/83	2.1
CGC7/83	3.1
CGC9/83	2.1
CGC10/83	3.2
CGC11/83	8.2
CGC12/83	10.2*
CGC13/83	1.2
CGC14/83	1.2
CGC16/83	2.2
CGC17/83	2.2
CGC19/83	3.2
CGC20/83	4.2
CGC21/83	1.2
CGC22/83	2.2
CGC23/83	1.2
CGC24/83	1.2
CGC25/83	2.2
CGC26/83	3.3
CGC27/83	2.3
CGC30/83	2.3

Bucks 1984 cull	
Specimen	**Age**
C1/84	2.11
C2/84	2.11
C3/84	0.10
C5/84	5.10
C7/84	2.11
C8/84	0.11
C9/84	5.11
C10/84	0.11
C11/84	1.11
C12/84	1.0
C14/84	7.0
C15/84	3.0
C16/84	4.0
C17/84	7.0
C19/83	3.0
C20/84	7.0
C21/84	3.1
C22/84	5.2
C23/84	4.1
C26/84	3.2
C27/84	9.2
C30/84	9.2

Bucks 1985 cull	
Specimen	**Age**
C1/85	3.10
C2/85	1.10
C3/85	0.10
C4/85	2.10
C5/85	2.10
C7/85	3.11
C8/85	2.11
C9/85	1.11
C10/85	3
C11/85	2
C18/85	4
C19/85	2.1
C20/85	2.1
C21/85	5.2
C22/85	1.2
C23/85	5.2
C24/85	3.2
C25a/85	3.2
C25b/85	2.2
C27/85	4.2
C28/85	4.3
C29/85	1.3
C30/85	1.3

(continues overleaf)

Table 4.1 continued.

Does 1983 cull	
Specimen	**Age**
C1/83	2
C2/83	5
C4/83	5
C5/83	3
C6/83	3
C7/83	5
C8/83	kid
C9/83	7
C13/83	1
C14/83	kid
C15/83	kid
C16/83	kid
C17/83	3
C18/83	5
C19/83	2
C20/83	9
C21/83	2
C22/83	4
C24/83	kid
C25/83	3
C26/83	1
C27/83	1
C28/83	5
C29/83	4
C30/83	kid

Does 1984 cull	
Specimen	**Age**
CG/84	1
CKCA/84	kid
C31/84	kid
C22/84	1.5
C59/84	5.8
CKCG/84	kid
C6/84	5
C42/84	2.7
CKCC/84	kid
CKC1/84	kid
C23/84	7.5
C1/84	6.5
CK/84	9
C48/84	2.7
C27/84	0.6
CV/84	2
CZ/84	7
CAB/84	13
C21/84	0.5
CO/84	4
C53/84	4.7
CU/84	11
C45/84	0.7
CA/84	8
CQ/84	1
CGI/84	3
CAA/84	3
CKCJ/84	kid

Does 1984 cull cont'd	
Specimen	**Age**
C36/84	1.6
CD/84	8
C4/84	6
CT/84	3
C18/84	10.5
C55/84	2.7
C3/84	1.5
CKCH/84	kid
CJ/84	3
CR/84	1
CW/84	2
C5/84	5
CKCK/84	kid
C56/84	3.7
C49/84	2.7
CKCF/84	kid
CM/84	9
C64/84	1.8
CEa/84	3
CEb/84	7
CN/84	7
CH/84	2
C7/84	8.5
CKCV/84	kid
CKCE/84	kid
CP/84	1
C32/84	1.6

(continues overleaf)

Table 4.1 continued.

Does 1985 cull			Does 1985 cull	
Specimen	**Age**		**Specimen**	**Age**
C1/85	4.4		C5/85	3.4
C6/85	1.4		C13/85	1.5
C26/85	0.5		C9/85	0.4
C44/85	7.7		C42/85	1.7
C40/85	3.7		C23/85	1.5
C45/85	0.7		C7/85	1.4
C35/85	3.6		C27/85	1.5
C11/85	1.5		C3/85	3.4
C32/85	10.6		C50/85	2.7
C23/85	1.5		C38/85	0.7
C38/85	0.7		C28/85	6.5
C22/85	2.5		C48/85	7.7
C14/85	0.5		C39/85	0.7
C29/85	0.6		C31/85	0.6
C49/85	1.7		C21/85	1.5
C12/85	2.5		C20/85	3.5
C46/85	0.7		C47/85	2.7
C37/85	1.6		C17/85	1.5
C33/85	8.6		C41/85	3.7
C10/85	0.4		C30/85	0.6
C24/85	1.5			

*The pattern of dental eruption observed revealed this specimen was a kid.

6 years (Table 4.1). The donated buck mandibles had been labelled CGC, to indicate their Craigellachie origin, and given a unique reference number incorporating the last two digits of their cull year at the end, for example CGC24/83 referred to a buck culled in 1983. Females were similarly labelled, but their reference numbers had been prefixed C to allow ease of distinction between the sexes.

4.2 Initial Cleaning Of The Sample

The mandibles were received in a partially defleshed state, and remaining soft tissues had become dehydrated and hardened due to warehouse storage. To secure loose molars, many mandibles had been covered in masking tape which had also hardened and had become firmly fixed to remaining soft tissues and exposed cortical bone. Consequently, prior to any further consideration, the material was cleaned. The masking tape was soaked with 100% industrial methylated spirits, which softened the tape and allowed it to be teased away from each mandible. All loose molars were removed, washed and stored in 100% industrial methylated spirits in individual, labelled glass jars. Mandibles were then defleshed using a modified version of the Davis & Payne (1992) maceration technique, as follows:

1) Each mandible was placed into a nylon mesh bag, this prevented mixing and loss of further dentition during processing. (Nylon bags were made from the legs and feet of old clean tights and stockings.)
2) Once bagged, the mandibles were simmered in water, which rehydrated and loosened the soft tissues. Eight bagged mandibles at a time were placed into a large pan with approximately 5 litres of water. The pan was stood on a hot plate and its contents allowed to simmer gently for an hour. The water was not allowed to boil, as this can cause dissolution of inorganic bone minerals, nor to heat up too rapidly, as this can cause bones and teeth to crack.
3) After simmering, the mandibles were allowed to cool, then removed from the nylon bags and rinsed in cool running water. Rinsing was conducted over a sieve to prevent loss of loose teeth and to catch any soft tissue that had become loose, so that it could be suitably disposed of (in bins designated for laboratory waste). Further pieces of soft tissue that were not fully separated from

bone were teased away with tweezers, but only if this could be done without damaging the bone surface. These procedures enabled the removal of the bulk, but not all, of the soft tissues.

4) The partially cleaned mandibles were then placed into individual 1 litre glass beakers and covered with a solution of 2 tablespoons of Papain (an enzyme which breaks down soft tissues) dissolved in 1 litre of warm water. The beakers were stood on a hot plate so that their temperature could be maintained at 40° C, the temperature at which the enzyme was most active. The solution in each jar was changed daily until all soft tissue had been removed. Then mandibles were then rinsed and allowed to air dry. Enzyme maceration is an unpleasant process and so was conducted in a fume cupboard, and surgical gloves and a disposable breathing mask were used.

Defleshing did lead to microscopic damage and removal of circumferential lamellae from the subperiosteal surface of some specimens. As Bell (1990; p. 91) noted, 'the irregular removal of periosteal bone occurs along natural cleaved fracture lanes and can be distinguished from a surface produced by osteoclastic resorptive activity, which is characterised by a scalloped profile that passes through and across secondary osteonal bone, irrespective of natural cleavage planes'. Such damaged surfaces were avoided when subsequent counting of histological features was conducted (below), and it should be noted that archaeological bone often exhibits similar destructive removal of circumferential lamellae (Bell, 1990), so the validity of developing an ageing technique that relies on its quantification is questionable in any case. In this study no link between circumferential lamellar bone (where intact) and age was identified (Chapter 5). Consequently, the accidental damage and removal of circumferential lamellae was not considered a serious problem, although it is accepted that it is not ideal.

The length of time necessary for complete soft tissue maceration varied with respect to mandible size and with the amount of soft tissue present. Smaller mandibles from kids tended to require only 2 changes of solution over 1½ to 2 days, but the more robust adult mandibles required up to 4 changes of solution over 3 days. Restrictions on available laboratory space meant that only 8 specimens at a

Table 4.2: The Selected Roe Deer Sample (N=72).

Specimen	Sex*	Year of Life	Specimen	Sex*	Year of Life
C3/84	B	1	C32/84	D	2
C3/85	B	1	C64/84	D	2
C8/84	B	1	C49/84	D	3
C10/84	B	1	C55/84	D	3
CGC13/83	B	2	C47/85	D	3
C30/85	B	2	C22/85	D	3
C11/84	B	2	C5/85	D	4
C9/85	B	2	C3/85	D	4
CGC5/83	B	3	C56/84	D	4
CGC9/83	B	3	C40/85	D	4
C25/85	B	3	C29/83	D	5
CGC25/83	B	3	C1/85	D	5
C15/84	B	4	C53/84	D	5
C10/85	B	4	CX/84	D	5
CGC10/83	B	4	C28/83	D	6
C26/84	B	4	C7/83	D	6
C18/85	B	5	C59/84	D	6
C16/84	B	5	C18/83	D	6
C23/84	B	5	C4/84	D	7
CGC20/83	B	5	C1/84	D	7
C22/84	B	6	C28/85	D	7
C21/85	B	6	CN/84	D	8
C23/85	B	6	C48/85	D	8
C9/84	B	6	CZ/84	D	8
C14/84	B	8	C9/83	D	8
C17/84	B	8	CA/84	D	9
C20/84	B	8	CD/84	D	9
CGC11/83	B	9	C7/84	D	9
C27/84	B	10	C33/85	D	9
C30/84	B	10	C20/83	D	10
C9/85	D	1	CK/84	D	10
C10/85	D	1	CM/84	D	10
C51/84	D	1	C18/84	D	11
C21/84	D	1	C32/85	D	11
C7/85	D	2	CU/84	D	12
C27/85	D	2	CAB/84	D	14

*B = bucks, D = does

time could be cleaned. Each batch of specimens took approximately 2 days to clean, which meant a total cleaning time of 10 weeks for the whole sample.

4.3 Selection Of Mandibles For Analysis

It was decided that a smaller sample with a more even spread of ages would be of greater study value than the whole sample with its bias in age towards younger animals. As far as possible four bucks and four does from each year of life were selected for analysis (Table 4.2). For bucks this proved possible from kids, in their first year of life, through to those in their sixth year of life, but there were none in their seventh year and just three in their eighth year, one in its ninth year and two in their tenth years. Four doe mandibles were available for each age class from those in their first year of life through to those in their sixth year of life, and in addition there were three mandibles from animals in their seventh year, four from those in their eighth and ninth years, three in their tenth year, two in their eleventh year, and one each in their twelfth and fourteenth years of life. Thus a smaller sample of seventy-two specimens, including forty-two does and thirty bucks, was selected for microscopic examination of age-related bone changes.

4.4 Bone Pathology

Specimens with bone pathology were also excluded from the sample selected for analysis, as several pathological conditions are known to disrupt the normal remodelling rates of bone (Kerley, 1965), for example Paget's disease increases bone turnover (Munzenberg *et al.*, 1971) and tumours are thought to stimulate bone resorption (Boyde *et al.*, 1986). The Craigellachie mandibles included two specimens with bony growths on their sides, and these were not considered for analysis. Some alveolar resorption was also noted in several specimens and, even though this was generally confined to an area around the base of the premolars well away from the proposed study area (under the 1st and 2nd molars) and it was recognised that Kerley (1965) had not detected abnormalities in remodelling rates from normal bone sections adjacent to those with pathological lesions, it was decided that the specimens showing alveolar resorption could not be considered for analysis. Gross examination of the mandibles for pathological lesions was supplemented by radiographic analysis with the Institute of Archaeology Faxitron machine. For the more robust adult specimens a voltage of 40kV for 30 seconds was found to provide the sharpest images. Kodak Industrex AX film was used. Exposure time was reduced by 5 seconds for the less dense juvenile material. Examination of the resulting X-rays did not reveal any signs of pathology in the remaining specimens. It took three weeks to x-ray all specimens, and process and analyse all x-rays.

4.5 Thin Sectioning

Initially following Kerley (1965) and others who have studied microscopic age changes in bone (Chapter 3), thin sections of bone were to be prepared from pieces removed from each of the mandibles in the sub-sample for histological examination under a polarising microscope. The optimum thickness required for bone thin sections is 75μm. In the event, thin section preparation proved much more problematic than anticipated and a variety of approaches was tested.

Frost (1958, p. 273) described his thin sectioning technique as "simple, cheap, rapid and reliable" and used it to produce 10 μm thick bone sections in less than 10 minutes. He removed pieces of bone (1 to 2mm thick) from main shafts, using a fine-toothed hack saw, and hand ground them on moistened carborundum abrasive paper. When a bone piece became too thin to handle, a grip was made by wrapping a strip of fresh carborundum paper around a microscope slide and pressing the slide against the top of the bone piece. Frost noted that bone sections ground to approximately 75μm thick appeared 'mottled white'. Once ground to the required thickness sections were rinsed, to remove loose bits of abraded bone, and left to dry at room temperature. Cleaned and dry sections were then mounted on microscope slides, ready for analysis.

Although appearing simple, several problems were encountered when practicing the Frost technique:

- The hack saw caused severe periosteal flaking of bone shafts. An Isomet slow speed diamond saw was used instead, but this slowed the procedure and it took up to 20 minutes to remove bone pieces from their shafts.

- The 'carborundum paper and microscope slide grip' was difficult to manipulate. The paper slid along and off the microscope slide, and the whole thing had to be constantly reassembled after just a few circular grinding movements.
- It was difficult to maintain even pressure on the grip, and several microscope slides and bone pieces were snapped or cracked.
- It was difficult to 'catch' bone pieces at the '75 μm mottled white stage', and over-grinding resulting in the loss of part of each bone section was common.

Even after much practice it still took almost an hour to produce a single bone section suitable for slide mounting, and not the 10 minutes Frost reported.

An alternative method of thin section preparation devised by the American company Buehler Limited (1988) was also tried. Buehler advise mounting bone pieces to microscope slides prior to hand grinding, and they supply a special holder which can be used to manipulate the microscope slide during the grinding. However, difficulty was experienced when trying to find an adhesive that would cement bone pieces to microscope slides and withstand the rigours of grinding. Various bonding agents were tried, including Loctite Superglue 3, Loctite glass bond glue and Lakeside cement glue. This latter glue required heating to a temperature of over 40° to make it soft and pliable before use, but this proved disastrous as rapid exposure to a hot substance caused bone pieces to crack. Loctite Superglue 3 and Loctite glass bond glue did join bone pieces to the microscope slides, but did not withstand grinding. A two-part epoxy resin adhesive was found to be more successful, but still half of all sections failed during final grinding. As it was the carborundum slurry which appeared to cause the glues to fail, abrasive papers requiring only limited lubrication were substituted during final grinding stages, and eventually thin sections were made, but each one required over 30 minutes grinding and success was not guaranteed.

It was felt that this methodology would, in any case, not be suitable for important and delicate archaeological specimens, so an alternative mode of microscopic analysis not requiring the use of thin sections was sought. This proved to be the use of the back-scattered electron detector in the scanning electron microscope.

4.6 The Use Of Back-Scattered Electron Imaging In The Scanning Electron Microscope For Examining Bone

Scanning electron microscope (SEM) images are formed by scanning an electron beam across the surface of a specimen, housed within an evacuated chamber. As the beam moves across the specimen, electrons penetrate it and scatter throughout it, to a depth of between 1 and 5 μm (depending on how the microscope is operated and the nature of the sample). Some of the electrons scattering through a specimen re-emerge at the surface as back-scattered electrons (BSE). These can be measured by detectors, whose electrical signals are converted into an image which is viewed on a television-like monitor. The more BSE that are detected, the brighter the resulting image.

The rate at which BSE escape is influenced by:

1. The composition (the mean atomic number and density of atoms) of the specimen volume probed by the electron beam: the denser (in bone the more heavily mineralised) a specimen is then the greater is the chance of BSE escape. In contrast, the less dense (in bone the less heavily mineralised) a specimen is then the less is the chance of BSE escape.
2. The topography (relief) of the specimen surface: "High points and ridges allow increased chance of BSE escape, and conversely, it is more difficult for BSE to escape if the beam enters the sample below the general level of its surface." (Bell *et al.*, 1991).

At any one time, any area of bone will contain differing proportions of non-Haversian canals, primary osteons, secondary osteons, fragmentary osteons and circumferential lamellae. Some of these features will have been formed when the original bone template was laid down, and others will represent areas of later remodelling, but all will have originally been formed in an unmineralised state. Mineralisation of fresh bone tissue or osteoid (Chapter 2) does not begin immediately after it has been deposited, and there is a maturation period of between 5 and 10 days before it commences. Different features of bone mineralise at differing rates and to differing degrees, and similar features

Plate 4.1: A Compositional Image Of A Cross Section Of A Roe Deer Mandible.

Most recently formed features appear darker.

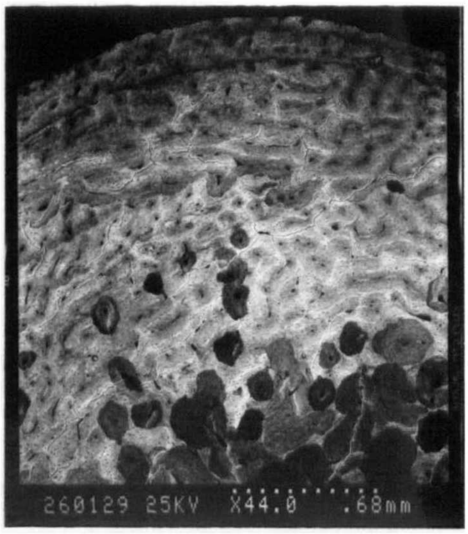
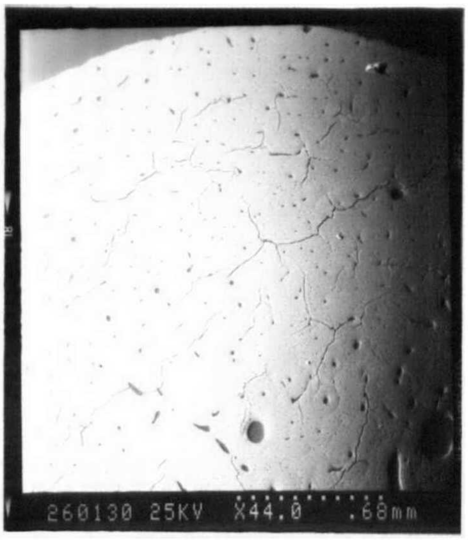


Plate 4.2: The Same Bone Field As Above, But Recorded As A Topographic Image.

Little detail can be seen because the surface is quite flat, but vascular canals and larger post-mortem damage holes are apparent because, although they are infilled with PMMA, they wear more quickly than adjacent areas of bone and, consequently, there is less BSE escape.



formed at different times are also more or less heavily mineralised. Consequently, the features within an area of bone will show differing levels of mineral density, which are reflected by their relative brightness in a BSE image. BSE imaging is thus an ideal way to study microscopic age changes in bone, but any relief in the specimen surface will also affect the rate at which BSE escape (above) and will therefore be apparent in the image. It is necessary to disentangle the effects of composition and relief in interpreting the image.

To reduce surface relief effects, specimens need to be made as flat as possible before examination. This is done by embedding in polymethylmethacrylate (PMMA), prior to sectioning and polishing (Boyde et al 1986). PMMA is a plastic that easily penetrates bone tissue and, once set, is one of the hardest materials available for impregnating specimens. Styrene is added to the mix to increase the stability of PMMA under electron bombardment. Embedding ensures that all holes within the bone, such as Haversian canals, are filled in. Once a flat surface is obtained a solid-state BSE detector can be used to produce images which emphasise the compositional/mineral density contrasts in the specimen. First, the specimen surface is aligned perpendicular to the electron beam and placed at a carefully selected distance from the detector to minimise topographic effects. Secondly, most solid-state detectors are divided into four sectors which can be individually switched in a way that also helps to minimise topography (a so called compositional image; Plate 4.1) or to maximise it (a topographic image; Plate 4.2). It is virtually impossible to remove all elements of topography from a polished surface because:

- PMMA wears more rapidly than bone (Bell *et al.*, 1991). Holes within the bone infilled with PMMA such as canals and osteocytic lacunae are more worn than adjacent bony areas and so appear as depressions within the surface.
- Bone containing collagen which is orientated parallel to a surface wears more than that which contains collagen orientated more perpendicularly to the surface (Boyde, 1984; Reid, 1986). Adjacent lamellae have collagen fibres of different predominant orientations and so wear at different rates, thus causing a slightly undulating surface.

- For a particular collagen orientation, less mineralised bone wears more rapidly than more heavily mineralised bone (Boyde & Jones, 1987). Less well mineralised secondary osteons and more recently formed areas of bone stand less proud than older more heavily mineralised areas of bone. The effects of the slight remaining topography therefore tend to reinforce the compositional effects.

Boyde & Jones were the first to investigate the practical application of using BSE imaging to study bone, including both normal and pathologic human bone, rabbit bone, calcified cartilage, (Boyde & Jones, 1983a) and dental tissues (Boyde & Jones, 1983b). They found that BSE imaging could be used to provide charge-free images, particularly in the converted back-scattered electron (CBSE) mode, of the mineral phase of bone, and that its sensitivity for the detection of atomic number contrast and the simplicity of sample preparation rendered it an ideal technique for the rapid acquisition of qualitative and comparative data from skeletal tissues. The compositional images mimicked the appearance of microradiographs and showed variations in mineral density at the level of the tissue surface and, in bone, features such as secondary osteons and cement reversal lines could be clearly identified. Later, Boyde *et al.* (1986) used BSE imaging in conjunction with a 'home made image analysis system', based on a Sharp MZ80K microcomputer, to quantify mineralisation levels and study the effects of disease processes on them, in bone. Mineralisation deficiencies were noted in woven bone biopsies from patients with fluorosis and in lamellar bone biopsies from patients with vitamin D resistant rickets. Abnormally high levels of mineralisation were seen in BSE images of two iliac crest biopsies from a 51 year old male with fibrogenesis imperfecta ossium and in a tibia biopsy from a 7 year old girl with osteogenesis imperfecta. In a summary of their findings, Boyde *et al.* (1986) noted that BSE imaging was a useful medium 'for studying features that were too difficult to study in the light microscope - such as details of the mineralising front and the pattern of collagen fibres in the walls of osteocyte lacunae'. Additionally, Boyde *et al.* (1990) used BSE imaging to study the microstructure of human cranial bone and cranial bone grafts. BSE imaging was used as it enabled detailed information about bone content, maturation and turnover to be gleaned from very small pieces of bone (as usually removed during biopsies). Both compositional and topographical images were recorded. The compositional images showed variation in the density of mineralisation of the bone tissue, and the topographic images allowed surface roughness, which also affects compositional image formation (see above), to be monitored. Bell (1990) used BSE imaging to study diagenesis (post-mortem decomposition through dissolution, precipitation, mineral replacement and recrystallisation) in archaeological normal and pathological human femoral and tibial sections. All specimens showed extensive sub-periosteal diagenetic changes, including both areas of excessive and poor mineralisation, which had obscured the characteristic morphology and density of adult bone. In contrast, deeper cortical regions were unaffected and retained much of their original bony structure, and features such as areas of remodelling, osteocytic lacunae and canaliculae could be clearly seen. This was in agreement with Samson & Branigan (1987; Chapter 3), who also noted greater structural preservation in deeper cortical regions of archaeological human bone than in sub-periosteal regions. In addition, Bell & Jones (1991) used BSE imaging to confirm the presence of Paget's disease (characterised by excessive and rapid turnover) in two adult inhumations from separate medieval cemeteries (St. Margaret *in cumbusto*, Norwich and Sandwell Priory). Later that year, Bell *et al.* (1991) used BSE imaging to study diagenesis in archaeological teeth and jaws, which had been recovered from five different soil-buried contexts (all cemeteries, dating from the Bronze Age through to the Medieval period) and one marine context (the wreck of the Mary Rose, Portsmouth, 1545 AD). BSE imaging revealed that:

- All the hard tissues, except enamel, showed diagenetic changes which marred their characteristic morphologies.
- In the soil buried jaws the pattern of diagenesis appeared to follow the vascular and cellular network of the bone, and it was orientated parallel to gross collagen direction.
- In the jaws recovered from the sea diagenesis ignored the vascular, cellular and collagenous network of bone, dentine and cement and, instead, "tissue destruction was located peripherally from the necks of the teeth crossing to the periosteal aspect of the alveolar crest and continuing peripherally around the entire distance of the subperiosteal bone of the mandible" (Bell *et al.*, 1991; pp. 175-6).

Bell *et al.* (1991; p. 181) concluded that "BSE imaging of the dental and supporting bony tissues proved a simple and effective method for investigation the changes in mineral density and morphology that accompany diagenesis". Finally, Boyde *et al.* (1991) used BSE imaging to study age-related mineral density changes in plastic embedded, polished blockfaces of bone removed from the sixth ribs of six female Soay sheep, ranging in known age from 0 to 11 years. The images revealed that:

- In both inner and outer parts of rib cortical bone, the mineral density of the tissue increased markedly during the first year of life.
- The mineral density distribution observed at 1 year was the same as that observed at sexual maturity (4 to 5 years).
- Bone turnover was greater in the inner cortical regions that the outer cortical regions, at every age studied.
- There was no continuous increase in bone mineral density in ewes of breeding age. This contrasted with earlier findings which had shown a continuous increase in bone mineral density in castrated male sheep from 0 to 9 years and in human males from 0 to 59 years.

4.7 Sample Preparation For The Scanning Electron Microscope

A slow speed Isomet diamond saw was used to remove 5mm thick slices of bone from each right mandibular ramus, in the region under the first and second molar. Each bone slice was embedded in polymethylmethacrylate (PMMA) with added styrene. A rapid method of PMMA embedding, developed by Sandra Bond of the Institute of Archaeology following Boyde (1986), was adopted and with kind permission this method is detailed below:

Bone sections were placed in:

1) A half and half solution of industrial methylated spirits and unwashed methylmethacrylate monomer for 4 hours
2) Unwashed methylmethacrylate monomer for 4 hours
3) A solution of 19 parts of unwashed methylmethacrylate monomer to 1 part styrene and a further 0.2% of the total solution volume of 2,2' Azobis 2-methylproprionitrile was also added. Bone pieces were left in this final solution for 24 hours at room temperature and for a further 5 days at 32°C, or until it polymerised (hardened).

The roe deer mandible slices were embedded in three batches, and the whole processes (including removal from the mandible and embedding) took four weeks.

After embedding bone slices were re-cut on the Isomet diamond saw in order to expose the required flat internal cross sectional surface, which was then polished using a Kent polishing machine. This machine has a rotating lap wheel onto which Hyprocel Pellon self-adhesive polishing mats are placed and diamond paste is put onto the mats. Two grades of diamond paste were used:

1) Grade 6 - PS - 42. This is a coarse paste which rapidly eroded the wheel marks caused by the Isomet saw.
2) Grade 1 - FS - 47. This is a finer paste which ensured as flat a surface as possible.

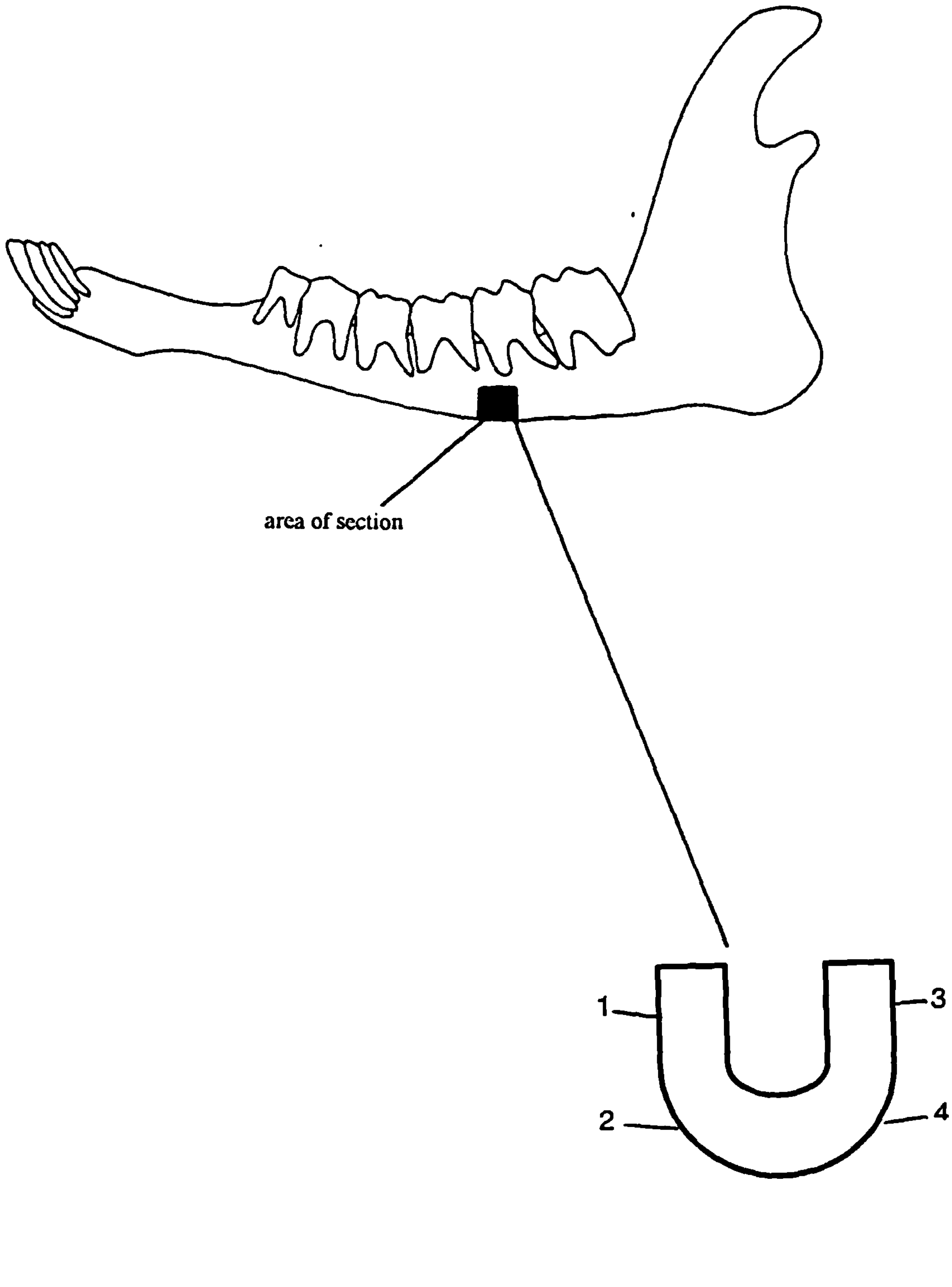
It took eight weeks to re-section and polish each of the 72 embedded bone slices.

It was never possible by this method to provide totally flat, topography-free surfaces, but with practice the surface undulations could be minimized, as could the evidence of scratches. The surface undulations result from uneven erosion of bone across the polished surface, due to differences in mineralisation and orientation of crystallites adjacent to the surface (above). It is accepted by Boyde (1984) that this cannot be avoided in routine preparation and the topography provided by the polishing in fact emphasises the underlying structure in the BSE image (above).

Polished specimens were rinsed in industrial methylated spirits, to remove traces of loose abraded bone and plastic, and were left to dry in air before being carbon coated using an Edward's carbon coater. Coating with carbon prevents specimens from charging in the SEM.

Preparation of the mandibular sections for analysis in the back-scattered electron detector was conducted with much greater ease and success than thin section production, although the new method was still somewhat lengthy and labour intensive. Removal of bone pieces from the seventy-two mandibles and embedding was conducted over a four week period, with the pieces being processed in three batches. Re-cutting, polishing and carbon-coating embedded bone pieces took an additional eight weeks. Specimens were then ready for scanning electron microscope analysis.

Figure 4.1: The Four Fields Of View Examined In The 72 Modern Roe Deer



4.8 Scanning Electron Microscope Operation

Each specimen was examined in an Hitachi 5-570 scanning electron microscope, fitted with a KE Developments solid-state back-scattered electron detector. Operating procedures were standardised as follows:

- Accelerating voltage - 25kV.
- Filament current - 100µA.
- Working distance (from the objective lens to the bone piece) - 15mm.
- Condenser lens 2 - between 3 and 4.
- Condenser lens 1 - reset for each specimen to produce the best possible image.
- Aperture - 3.

These specifications gave the best atomic number contrast in compositional images. Magnification was also standardised at x 44, this produced a rectangular field of view, measuring 2.5mm by 2.0mm at the level of the bone surface. Initial observations of the sections, in the back-scattered electron detector, did allow images to be produced in which all the histological features noted by Kerley (1965) as age-related in humans (circumferential lamellar bone, non-Haversian canals and secondary and fragmentary osteons). Two fields on both the lower lateral and lower medial sides of each mandibular section were examined (Figure 4.1). A camera attached to the SEM was used to record a photograph of each selected field, and the top edge of each photograph was positioned to lie just outside, but parallel, to the periosteal edge of each bone piece. As histological structure is known to be similar between adjacent areas of midshaft cortical bone (Kerley, 1965; Samson & Branigan, 1987), a degree of subjectivity was afforded in the selection of microscopic fields, and only those areas away from any "gross obscurity and cracking" (Aiello & Molleson, 1993; p. 691) were included. Histological features were quantified from the photographs (below) and not directly from the SEM monitor, because the quality of the images was better and because the flicker of the monitor screen caused difficulties. An extra photograph of one field per bone specimen was taken, to monitor unremoved topographical effects - however, no discrepancies were identified. Not all photographs 'worked' the first time - the BSE detector signal processor was difficult to adjust and images were not as always as sharp as anticipated. Some specimens had to be re-photographed several times before a clear image in which features could be clearly and confidently identified were produced. Photographs were printed at double the size of the negatives on Ilford A5, Grade 2 black and white photographic paper.

4.9 Recording Histological Features

The photographs showed rectangular fields of view but, to match the methods of Kerley (1965; 1969; 1970) and others (Singh & Gunberg, 1970; 1971; Thompson, 1979; Samson & Branigan, 1987; Ericksen, 1991), a circular field of view was defined inside them. This was produced by marking a circle with a diameter of 9.89cm on a sheet of acetate, and the upper edge of the acetate sheet was laid along the periosteal edge of the cross sectional surface shown in each photograph in turn and positioned to cover as much of the sample as possible. The acetate field delimited a field of view measuring $2.5mm^2$ at the level of the bone section, and although this was larger than Kerley's (1978) recalibrated field size of $2.06mm^2$ it was similar to that of Aiello & Molleson (1993), of $2.46mm^2$. The size of field selected in this study did not confine analysis of histological structure to the outer third cortex of bone, the area where Kerley (1965; p. 151) reported in humans that "the structural changes normally associated with age seemed better spaced to cover the total life span .... than in the middle or inner thirds", but extended over the middle third and most of the inner third of each bone section. In the present study preliminary observations of the roe mandible sections revealed that there appeared to be little age related structural change in the outer third cortex and that bone turnover and the formation of secondary osteons was generally confined to the mid- and inner-cortical thirds. These findings were in line with those of Boyde *et al.*, (1991) who reported greater bone turnover in the inner cortex than the outer cortex in Soay sheep, and are of greater relevance to the study of archaeological bone where greater structural preservation has been reported in the inner cortical regions (Samson & Branigan, 1987; Bell 1990).

Each of the four circular photographic fields per specimen was examined for the presence of those histological features shown by Kerley (1965) to be aged related, including:

- The number of secondary osteons.

- The number of fragmentary osteons.
- Circumferential lamellar bone area.
- The number of non-Haversian canals.

As in Kerley (1965) and in Aiello & Molleson (1993), secondary osteons were recorded as complete if their central canal was untouched by remodelling, and they and non-Haversian canals on the periphery of the field were recorded if all of their central canal lay within the field. However, it proved extremely difficult to record fragmentary osteons; in specimens that showed few fragmentary osteons they were easily identified as they were usually only partially obscured/fragmented by a single secondary osteon, but in specimens with greater degrees of remodelling, individual fragments were much less easily isolated, and it was extremely difficult to be sure of where one fragment stopped and another began. Consequently, counts of fragmentary osteons were considered to be unreliable and were abandoned. This was in line with Singh & Gunberg (1970) who noted that most interstitial bone (that between secondary osteons) consisted entirely of fragmentary osteons which were difficult to isolate and count. A waterbased pen was used to mark off secondary osteons and non-Haversian canals on an overlying acetate sheet as they were counted. The sheet had a 1mm grid copied onto it, which allowed the area of circumferential lamellar bone to be estimated by counting the number of grid squares it occupied. Once all the features present in a photograph had been recorded the sheet was wiped clean and transferred to the next photograph. Points were marked on each photograph to indicate the exact location of each field. In this way, a total of twelve variables were recorded for each of the seventy-two roe deer mandibles, including:

1. The number of secondary osteons in position 1
2. The number of secondary osteons in position 2
3. The number of secondary osteons in position 3
4. The number of secondary osteons in position 4
5. Circumferential lamellar bone area in position 1
6. Circumferential lamellar bone area in position 2
7. Circumferential lamellar bone area in position 3
8. Circumferential lamellar bone area in position 4

9. The number of non-Haversian canals in position 1

10. The number of non-Haversian canals in position 2

11. The number of non-Haversian canals in position 3

12. The number of non-Haversian canals in position 4

The total and average counts of secondary osteons, circumferential lamellar bone area and non-Haversian canals were also calculated to provide an additional six variables for each specimen:

13. The total number of secondary osteons per specimen

14. The average number of secondary osteons per specimen

15. The total circumferential lamellar bone area per specimen

16. The average circumferential lamellar bone area per specimen

17. The total number of non-Haversian canals per specimen

18. The average number of non-Haversian canals per specimen

Initially, recording took up to thirty minutes for each photograph, and those photographs with a more diverse range of histological features took longer to count than those that mainly consisted of a single feature such as non-Haversian canals. However, with practice counting time was reduced to a maximum of fifteen minutes per photograph. Twelve photographs were counted each day and the whole counting procedure took four weeks to complete. Although counting from the photographs with the naked eye was quite tiring it was found to be much easier than studying the histological structure of thin sections down the eyepieces of a polarising microscope, and the use of the pen and acetate sheet meant that features were neither ignored nor mistakenly counted twice. The photographs also provided a permanent record of the bone fields examined and, consequently, permitted a study of observer error, in which half of the specimens were re-counted at a later date, and ten of these were also further counted by an independent observer (Chapter 5.1).

4.10 In Summary

Although the eventual procedures adopted for isolating and recording the selected histological features in the mandibular cross sections were quite simple they were extremely lengthy and labour intensive, and it took approximately a year to process all the selected mandibles. The time taken for each processing stage is listed below:

- Initial Cleaning -ten weeks.
- X-raying - three weeks.
- Removing a cross section from each mandible and plastic embedding - four weeks.
- Re-cutting each section, polishing and carbon coating - eight weeks.
- Taking and processing all three hundred and sixty photographs (four compositional and one topographic photograph for each of seventy-two specimens) -approximately twenty-six weeks (technical problems with the back-scattered detector were encountered).
- Count of histological features - four weeks.
- Counts for study of observer error (below) - three weeks.

CHAPTER 5 STATISTICAL ANALYSIS OF THE HISTOLOGICAL COUNTS

All bone histology counts and cement layer ages were entered into an SPSS data file (Norušis, 6.1, *SPSS Guide to Data Analysis*). SPSS is a standard programme that enabled the rapid computation of all statistics necessary for this investigation of relationships between bone histology and cement layer age in roe deer.

5.1 Observer Error

Counting features (at both the macroscopic and microscopic level) involves an element of personal interpretation, and so estimates of intra- and inter-observer error are necessary to evaluate the applicability of the adopted recording procedure (Charles *et al.*, 1986).

5.1.1 INTRA-OBSERVER ERROR

To enable a study of intra-observer error, thirty-six specimens were selected for recounting. Recounts were conducted in the manner described in Chapter 4.9, but without access to earlier results. No attempt was made to record fragmentary osteons. The new counts were used to generate new total and average feature counts for each specimen. At least one specimen from each year of life for both bucks and does was deliberately selected for recounting (Table 5.1). This was done to see if all combinations of feature counts could be accurately replicated as, preliminary observations of the photographs revealed that, younger specimens showed greater proportions of non-Haversian canals and older specimens showed greater proportions of secondary osteons.

5.1.1.1 t-tests

t-tests (Norušis, Chapter 13) were conducted to compare the mean values of each series of original and second counts. That that is, the mean value of original secondary osteon counts in position 1 was compared with the mean value of the second series of secondary osteon counts in position 1, and so on for all counts (Table 5.2).

Figure 5.1: Key To Feature Count* Abbreviations Used In Chapter 5 Tables

Feature Count	Definition
OST1	Secondary osteon count in position 1
OST2	Secondary osteon count in position 2
OST3	Secondary osteon count in position 3
OST4	Secondary osteon count in position 4
ALLOST	Total secondary osteon count
AVEOST	Average secondary osteon count
CIRC1	Circumferential lamellar bone area in position 1
CIRC2	Circumferential lamellar bone area in position 2
CIRC3	Circumferential lamellar bone area in position 3
CIRC4	Circumferential lamellar bone area in position 4
ALLCIRC	Total circumferential lamellar bone area
AVECIRC	average circumferential lamellar bone area
N-HAV1	Non-Haversian canal count in position 1
N-HAV2	Non-Haversian canal count in position 2
N-HAV3	Non-Haversian canal count in position 3
N-HAV4	Non-Haversian canal count in position 4
ALLN-HAV	Total non-Haversian canal count
AVEN-HAV	Average non-Haversian canal count

Table 5.1: Specimens Selected For Recounting

Specimen	Sex	Age (year of life)	Specimen	Sex	Age (year of life)
C3/84	B	1	C47/85	D	3
C3/85	B	1	C5/85	D	4
CGC13/83	B	2	C40/85	D	4
C11/84	B	2	C29/83	D	5
CGC5/83	B	3	C28/83	D	6
C25/85	B	3	C7/83	D	6
C15/84	B	4	C4/84	D	7
C10/85	B	4	C1/84	D	7
C18/85	B	5	CN/84	D	8
C22/84	B	6	C48/85	D	8
C21/85	B	6	C9/83	D	8
C23/85	B	6	CD/84	D	9
C14/84	B	8	C7/84	D	9
CGC11/83	B	9	C20/83	D	10
C27/84	B	10	C18/84	D	11
C10/85	D	1	C32/85	D	11
C27/85	D	2	CU/84	D	12
C49/84	D	3	CAB/84	D	14

<u>Key</u>

B - Bucks

D - Does

Table 5.2: t-tests Applied To The Original And Repeat Counts (n=36)

Feature Count*	Original Mean Count	Second Mean Count	Difference Between Mean Counts	*P*
OST1	33.00	32.80	-0.20	0.97
OST2	39.30	38.40	-0.90	0.90
OST3	31.60	31.60	0.00	0.99
OST4	36.40	35.20	-1.20	0.83
ALLOST	140.10	134.80	-5.30	0.81
AVEOST	35.80	34.10	-1.70	0.76
CIRC1	3.61	3.42	-0.19	0.90
CIRC2	0.42	0.44	+0.02	0.95
CIRC3	1.08	0.39	-0.69	0.36
CIRC4	1.31	1.31	0.00	0.99
ALLCIRC	6.50	6.10	-0.40	0.89
AVE CIRC	1.59	1.67	+0.08	0.90
N-HAV1	54.30	53.10	-1.20	0.92
N-HAV2	75.90	75.40	-0.50	0.98
N-HAV3	95.20	84.50	-10.07	0.51
N-HAV4	55.40	56.20	+0.80	0.95
ALLN-HAV	281.00	272.00	-9.00	0.87
AVEN-HAV	70.00	68.00	-2.00	0.89

Secondary Osteons

- Although some differences between mean counts were noted they were not significant (P>0.75).
- The repeat mean counts in positions 1, 2 and 4, and the repeat total and average mean counts were smaller than the original mean counts.
- There was no difference between the means of the position 3 counts.
- The largest difference between means was 5.30 (P=0.81), for the total counts.

Circumferential Lamellar Bone Area

- Although some differences between mean counts were noted they were not significant.
- For the difference in means of the position 3 counts P=0.36.
- For the difference in means for all other counts P>0.89.
- The repeat mean counts in positions 1 and 3, and the repeat total mean count were smaller than corresponding original mean counts.
- The repeat mean count in position 2, and the repeat average mean count were larger than corresponding original mean counts.
- There was no difference between the means of the position 4 counts.
- The largest difference between means was 0.69 (P=0.36), for the position 3 counts.

Non-Haversian Canals

- Although some differences between mean counts were noted they were not significant.
- For the difference in means of the position 3 counts P=0.51.
- For the difference in means of all other counts P>0.86.
- The repeat mean counts in positions 1, 2 and 3, and the repeat total and average mean counts were smaller than the original corresponding mean counts.
- The repeat mean count in position 4 was larger than the original mean count in position 4.
- The largest difference between means was 10.07 (P=0.51), for the position 3 counts.

5.1.1.2 Scatterplots

Scatterplots were used to allow the pattern of differences between each pair of counts to be investigated (Keiser, 1990). The difference between corresponding values in each pair of counts was plotted against their mean. That is, the difference between the original count of non-Haversian canals in position 1, specimen 1 and the second count of non-Haversian canals in position 1, specimen 1 was plotted against the mean value of the original count of non-Haversian canals in position 1, specimen 1 and second count of non-Haversian canals in position 1, specimen 1, and so on for all counts in all specimens (Figures 5.2 to 5.19). Differences were not plotted if the value of each pair of counts was 0.

Secondary Osteons

- Less than a third of all paired counts showed differences.
- Some second counts were slightly larger than original counts, but more tended to be slightly smaller than original counts.
- There was no pattern or trend to the differences between counts.

Circumferential Lamellar Bone Area

- Less than a third of all paired counts showed differences.
- Some second counts were slightly larger than original counts and some were slightly smaller.
- For the average counts, the difference between counts increased as their mean value increased, but these differences had been shown to be insignificant (Table 5.2).
- For all other circumferential lamellar bone area counts, no pattern or trend to the differences between counts was isolated.

Non-Haversian Canals

- Just over a third of all paired counts showed differences.
- Some second counts were slightly larger than original counts, but more tended to be slightly smaller than original counts.

Figure 5.2: Scatterplot To Highlight Trends In Intra-Observer Error For Secondary Osteon Counts In Position 1

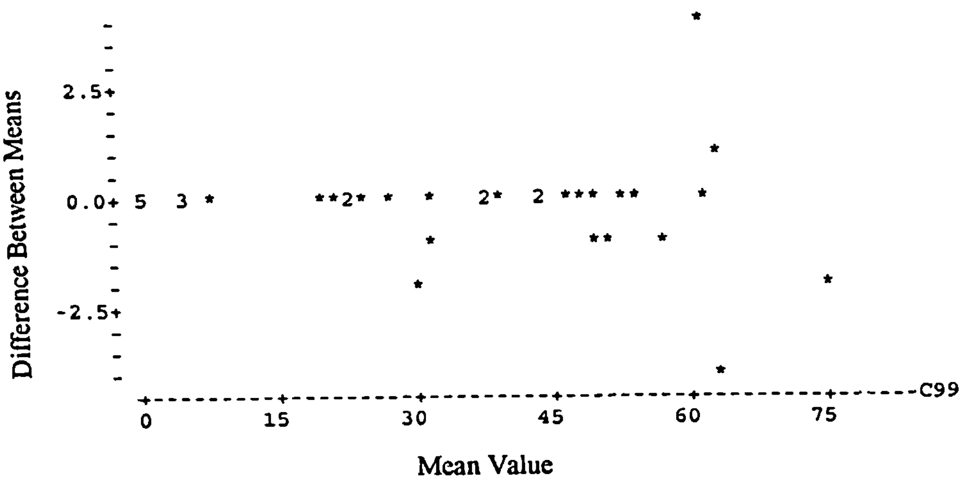


Figure 5.3: Scatterplot To Highlight Trends In Intra-Observer Error For Secondary Osteon Counts In Position 2

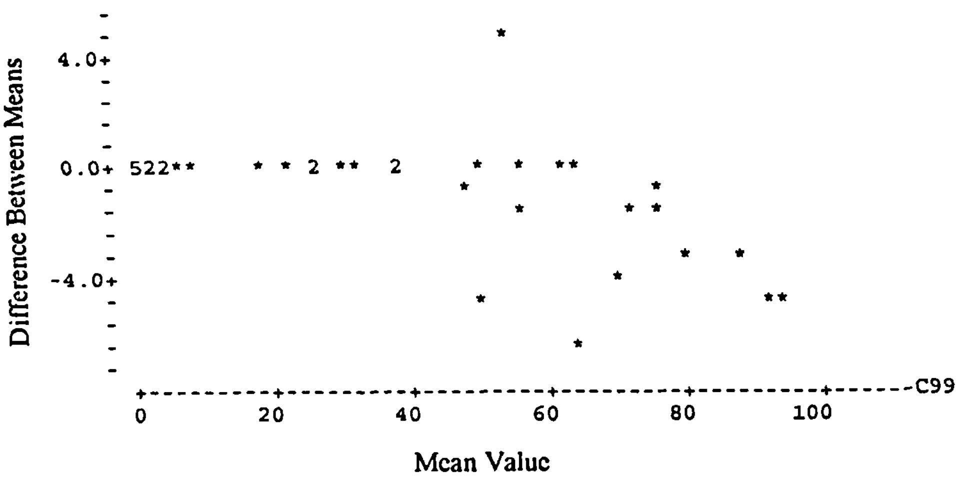


Figure 5.4: Scatterplot To Highlight Trends In Intra-Observer Error For Secondary Osteon Counts In Position 3

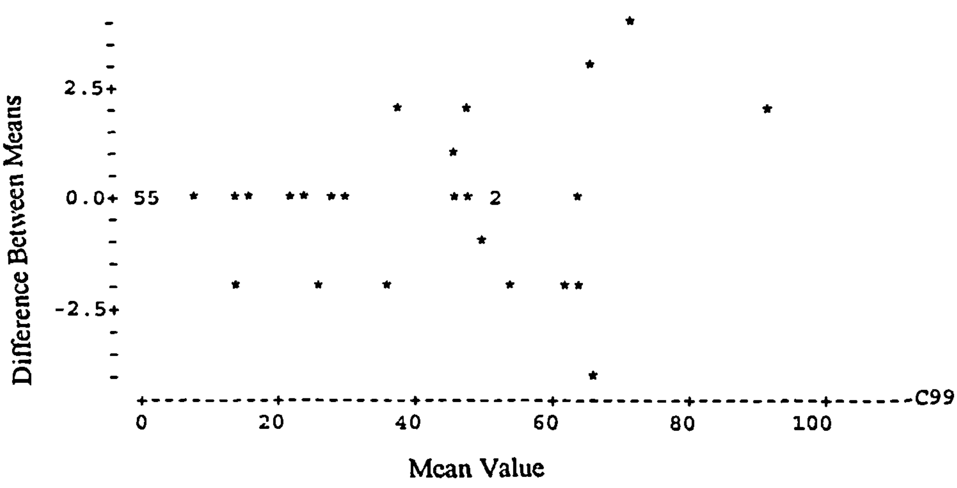


Figure 5.5: Scatterplot To Highlight Trends In Intra-Observer Error For Secondary Osteon Counts In Position 4

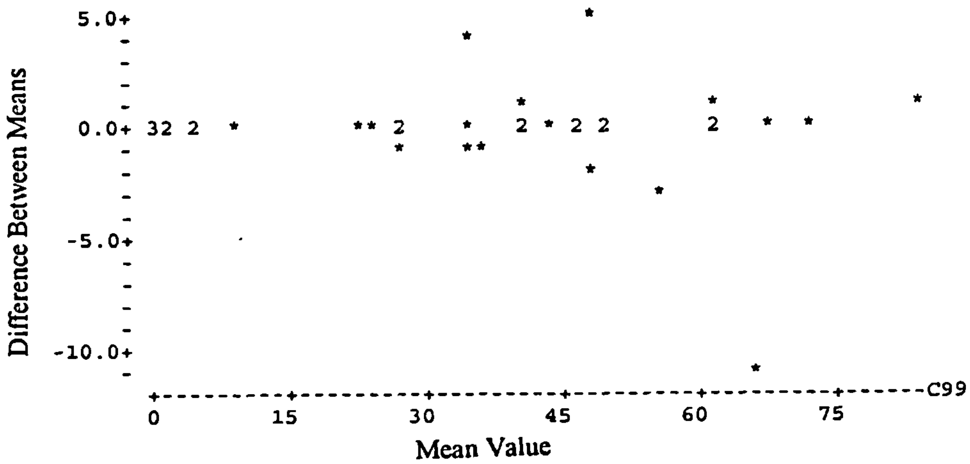


Figure 5.6: Scatterplot To Highlight Trends In Intra-Observer Error For Total Secondary Osteon Counts

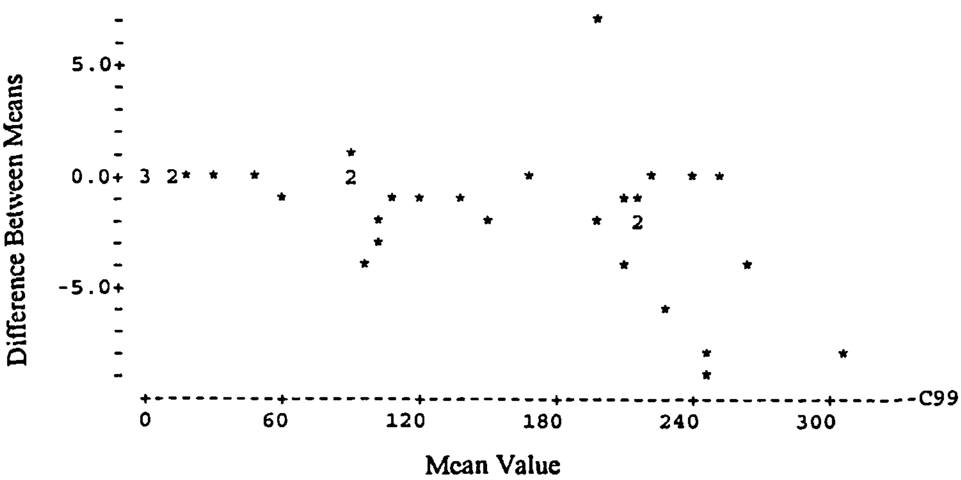


Figure 5.7: Scatterplot To Highlight Trends In Intra-Observer Error For Average Secondary Osteon Counts

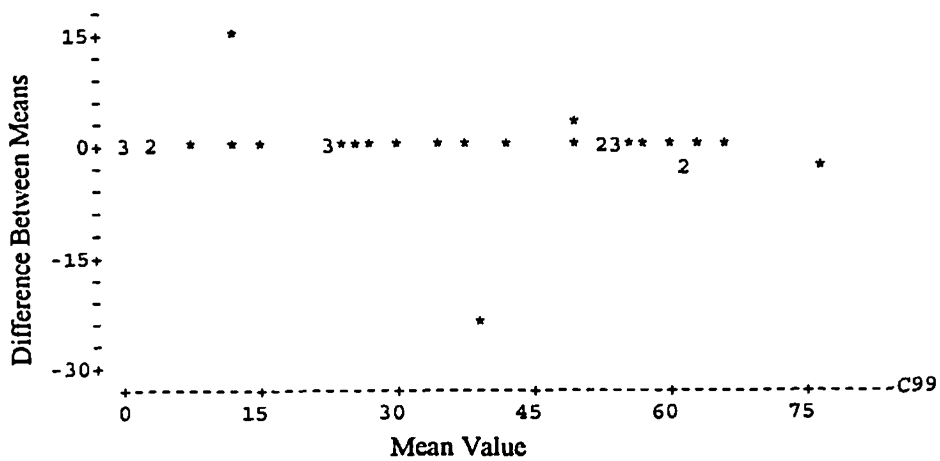


Figure 5.8: Scatterplot To Highlight Trends In Intra-Observer Error For Circumferential Lamellae Area In Position 1

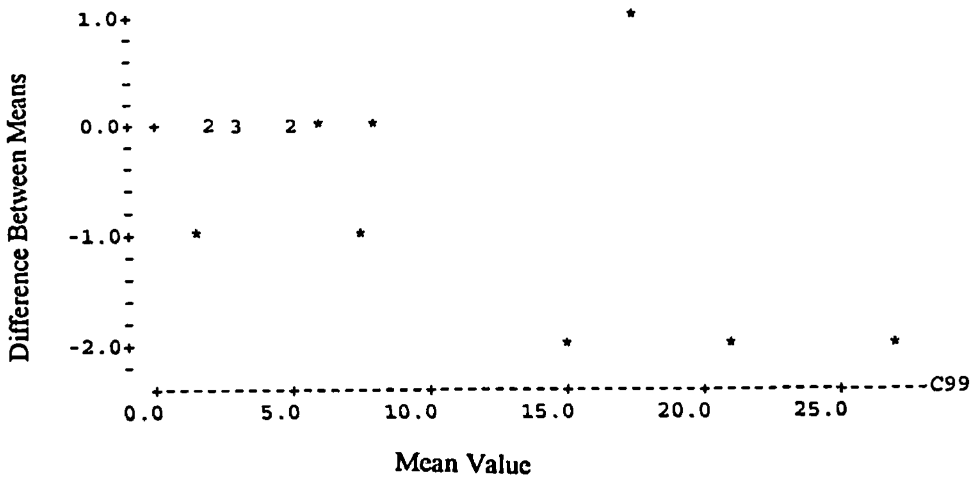


Figure 5.9: Scatterplot To Highlight Trends In Intra-Observer Error For Circumferential Lamellae Area In Position 2

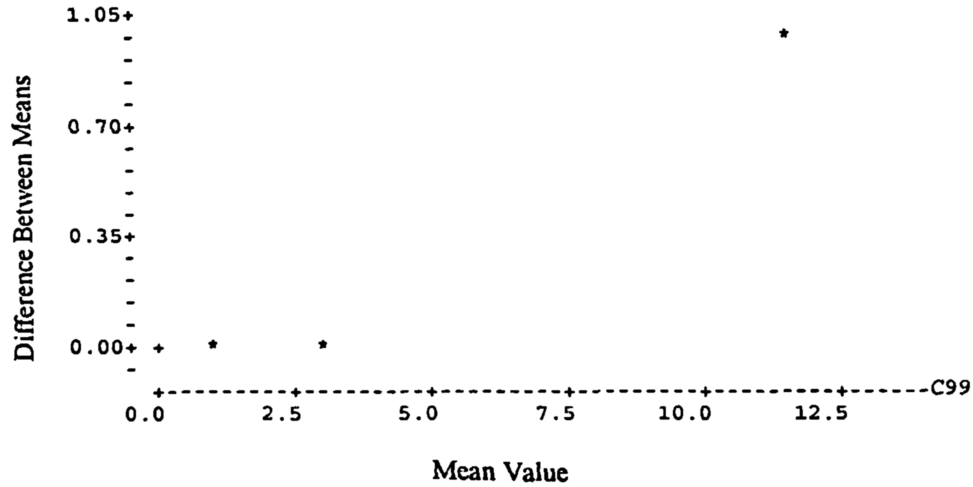


Figure 5.10: Scatterplot To Highlight Trends In Intra-Observer Error For Circumferential Lamellae Area In Position 3

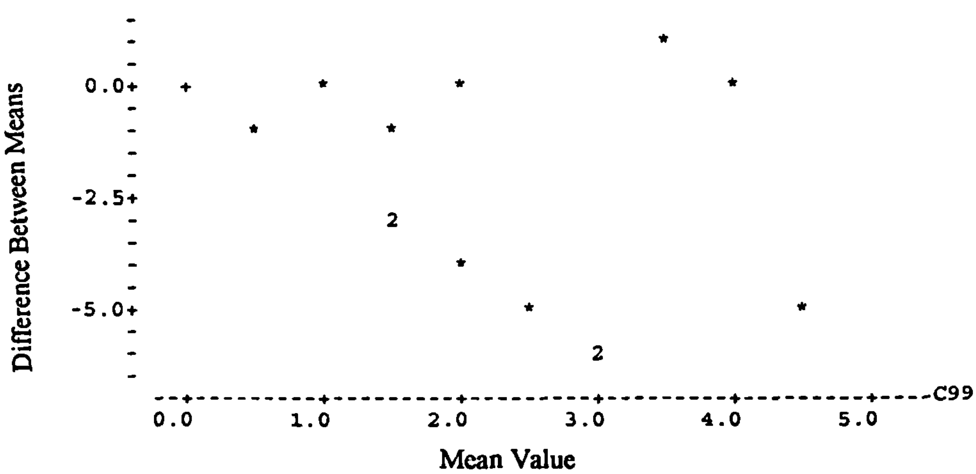


Figure 5.11: Scatterplot To Highlight Trends In Intra-Observer Error For Circumferential Lamellae Area In Position 4

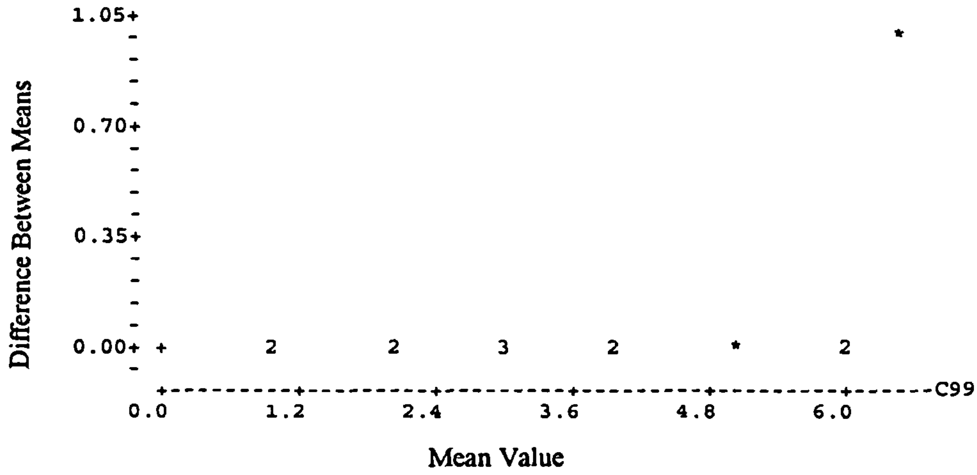


Figure 5.12: Scatterplot To Highlight Trends In Intra-Observer Error For Total Circumferential Lamellae Area

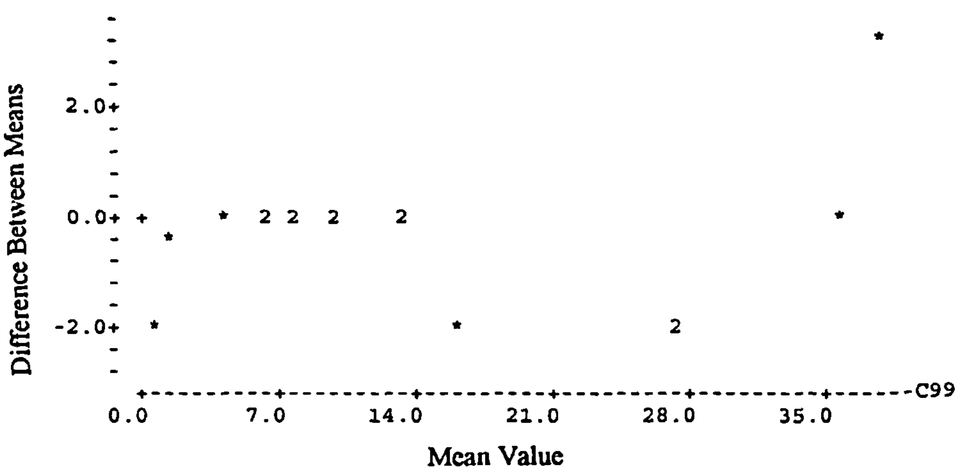


Figure 5.13: Scatterplot To Highlight Trends In Intra-Observer Error For Average Circumferential Lamellae Area

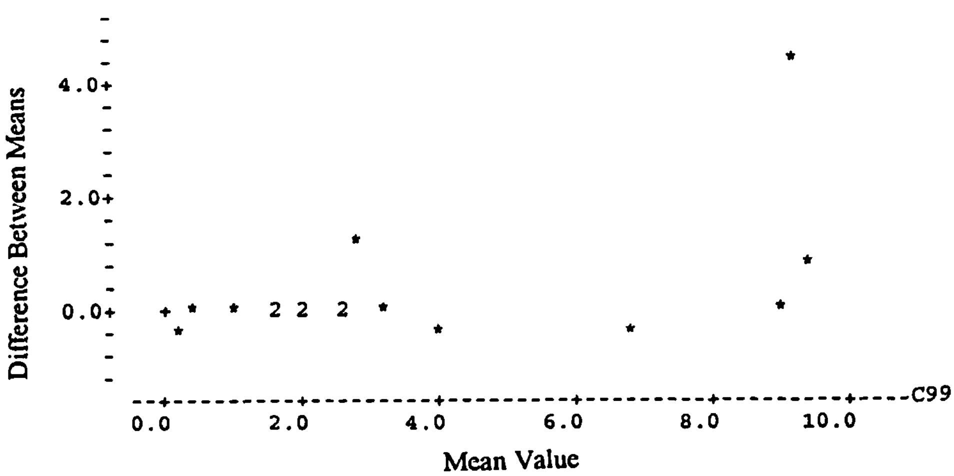


Figure 5.14: Scatterplot To Highlight Trends In Intra-Observer Error For Non-Haversian Canal Counts In Position 1

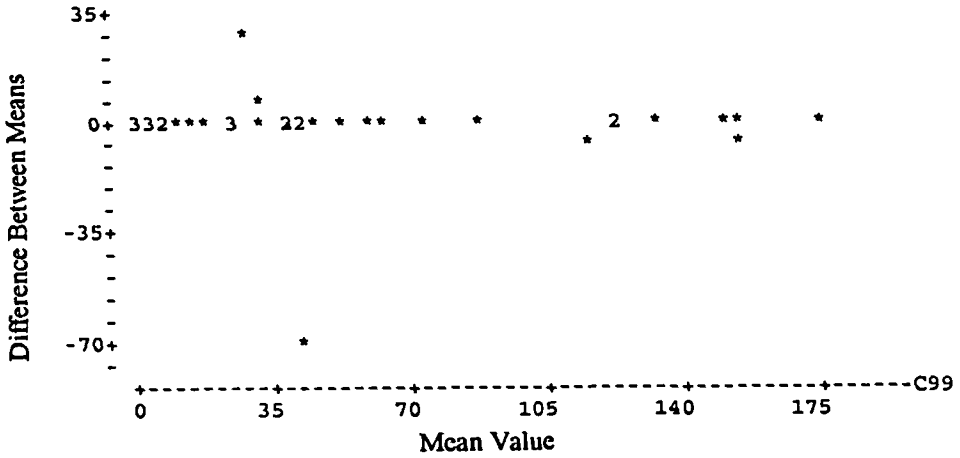


Figure 5.15: Scatterplot To Highlight Trends In Intra-Observer Error For Non-Haversian Canal Counts In Position 2

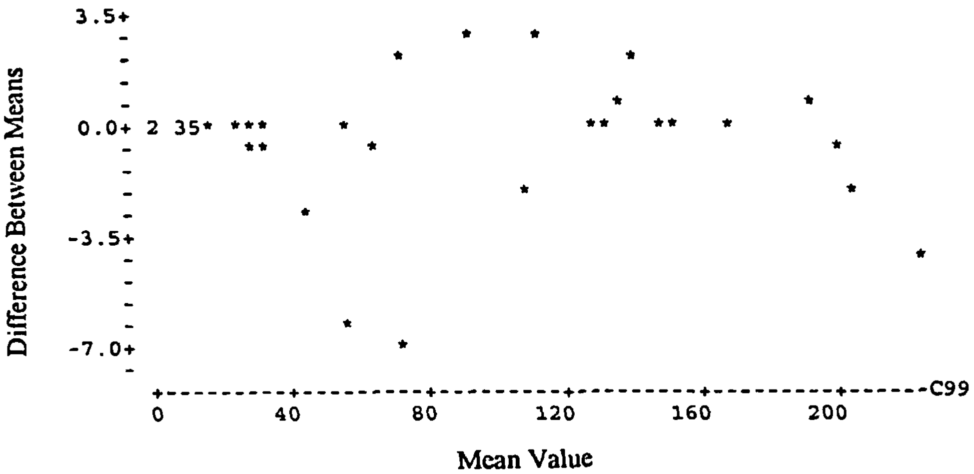


Figure 5.16: Scatterplot To Highlight Trends In Intra-Observer Error For Non-Haversian Canal Counts In Position 3

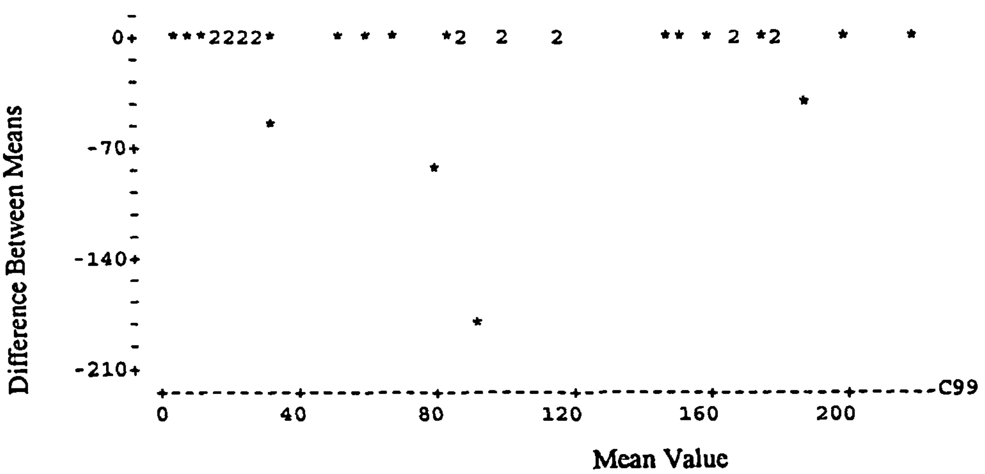


Figure 5.17: Scatterplot To Highlight Trends In Intra-Observer Error For Non-Haversian Canal Counts In Position 4

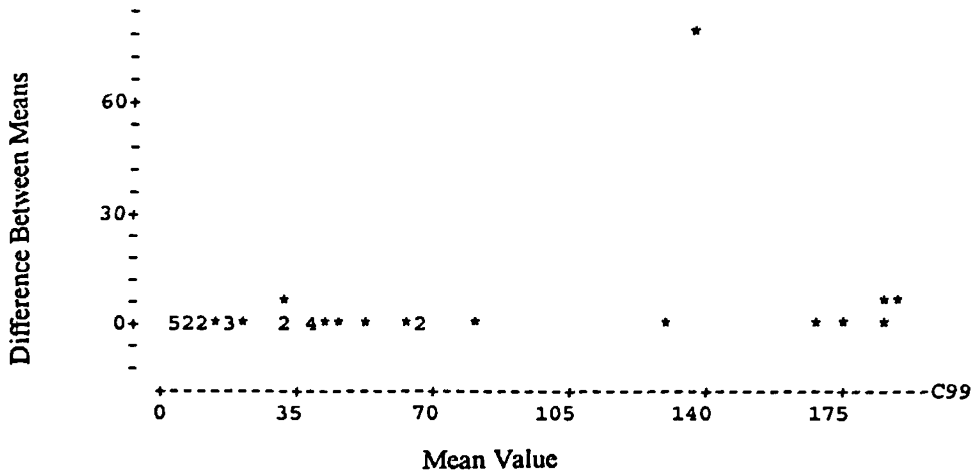


Figure 5.18: Scatterplot To Highlight Trends In Intra-Observer Error For Total Non-Haversian Canal Counts

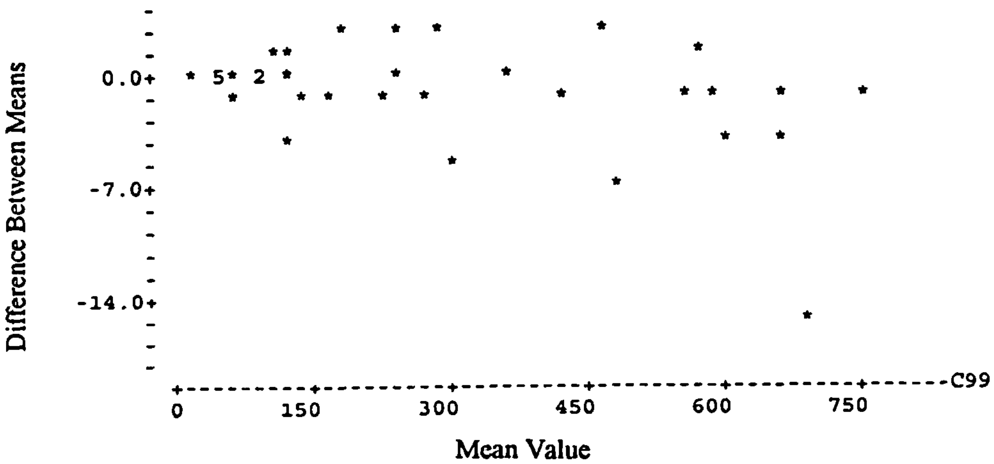
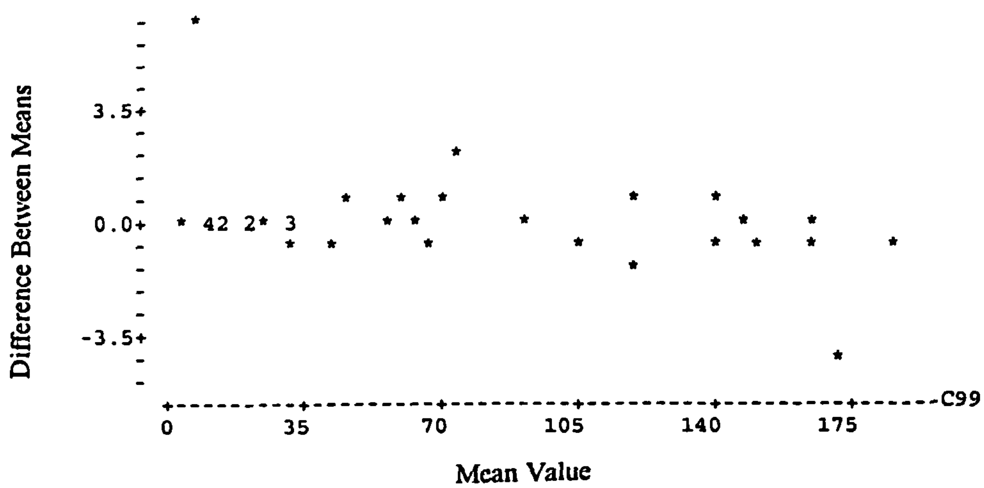


Figure 5.19: Scatterplot To Highlight Trends In Intra-Observer Error For Average Non-Haversian Canal Counts



- No pattern or trend to the differences between counts was isolated.

5.1.1.3 In Summary

- t-tests showed that there were no significant differences between the paired counts of the original observer.
- The scatterplots showed that, although there were differences between some individual counts in each paired series, there were no specific intra-observer error trends and that features were not consistently under or over recorded during counting. However it was noted that, where a difference was present, second counts were generally smaller than original counts.

5.1.2 INTER-OBSERVER ERROR

To allow a study of inter-observer error, ten specimens (Table 5.3) were selected for counting by an independent observer (B). Counting was, again, conducted in the manner described in Chapter 4.9, but without access to earlier results. No attempt was made to record fragmentary osteons. Observer B's count were used to generate new total and average feature counts for each of the ten specimens.

5.1.2.1 t-tests

t-tests were used to compare the mean values of each series of original counts and observer B's counts (Table 5.4).

Secondary Osteons

- Although differences between mean counts were noted they were not significant (P>0.80).
- All observer B's mean counts were smaller than the original mean counts.
- The largest difference between means was 7.00 (P=0.87), for the total counts.

Circumferential Lamellar Bone Area

- Although some differences between mean counts were noted they were not significant (P>0.84).

Table 5.3: Specimens Selected For Counting By Observer B

Specimen	Sex	Age (year of life)	Specimen	Sex	Age (year of life)
C3/84	B	1	C27/85	D	2
CGC5/83	B	3	C5/85	D	4
C18/85	B	5	C28/83	D	6
C14/84	B	8	CN/84	D	8
C27/84	B	10	CU/84	D	12

Key

B - Bucks

D - Does

Table 5.4: t-tests Applied To Original and Observer B's Counts (n=10)

Feature Count*	Original Mean Count	Observer B Mean Count	Difference Between Mean Counts	*P*
OST1	34.40	32.40	-2.00	0.83
OST2	33.40	32.10	-1.30	0.92
OST3	26.80	26.60	-0.20	0.99
OST4	31.40	28.70	-2.70	0.81
ALLOST	126.80	119.80	-7.00	0.87
AVEOST	31.70	30.00	-1.70	0.86
CIRC1	2.30	2.20	-0.10	0.94
CIRC2	0.40	0.50	+0.10	0.85
CIRC3	3.30	3.10	-0.20	0.95
CIRC4	1.60	1.60	0.00	1.00
ALLCIRC	7.60	7.40	-0.20	0.97
AVE CIRC	1.89	1.85	-0.04	0.97
N-HAV1	46.70	44.80	-1.90	0.92
N-HAV2	88.70	85.60	-3.10	0.93
N-HAV3	94.60	86.60	-8.00	0.77
N-HAV4	60.40	60.20	-0.20	1.00
ALLN-HAV	290	283	-7.00	0.94
AVEN-HAV	72.60	70.70	-1.90	0.94

- Observer B's mean counts in positions 1 and 2, and the mean total and average counts were smaller than the original corresponding mean counts.
- Observer B's mean count in position 2 was larger than the original corresponding mean count.
- There was no differences between the means of the position 4 counts.
- The largest difference between means was 0.20 (P=0.97), for the average counts.

Non-Haversian Canal Counts

- Although some differences between mean counts were noted they were not significant (P>0.76).
- All of observer B's mean counts were smaller than the original mean counts.
- The largest difference between means was 8.00 (P=0.77), for the position 3 counts.

5.1.2.2 Scatterplots

Again, the differences between each pair of counts in each original series and observer B's counts were plotted against their mean (Figures 5.20 to 5.37) to allow the pattern of any biases in counting procedures to be detected. Differences were not plotted if the value of each pair of counts was 0.

Secondary Osteons

- Just over a half of all paired counts showed differences.
- Some of observer B's counts were slightly larger than the original counts, but more tended to be slightly smaller.
- There was no overall pattern or trend to the differences between counts.

Circumferential Lamellar Bone

- Less than a quarter of all paired counts showed differences.
- There was an even split between the number of observer B's counts that were slightly smaller and slightly larger than the original counts.
- There was no overall pattern or trend to the differences between counts.

Figure 5.20: Scatterplot To Highlight Trends In Inter-Observer Error For Secondary Osteon Counts In Position 1

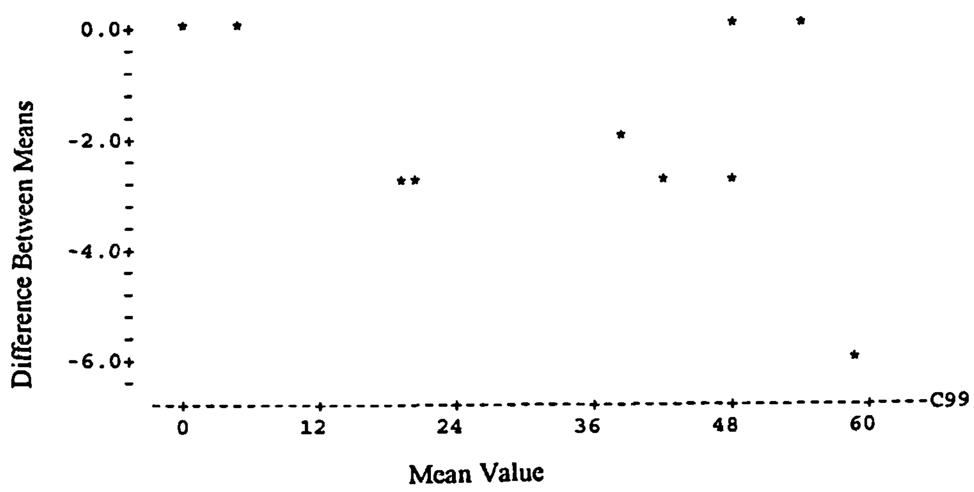


Figure 5.21: Scatterplot To Highlight Trends In Inter-Observer Error For Secondary Osteon Counts In Position 2

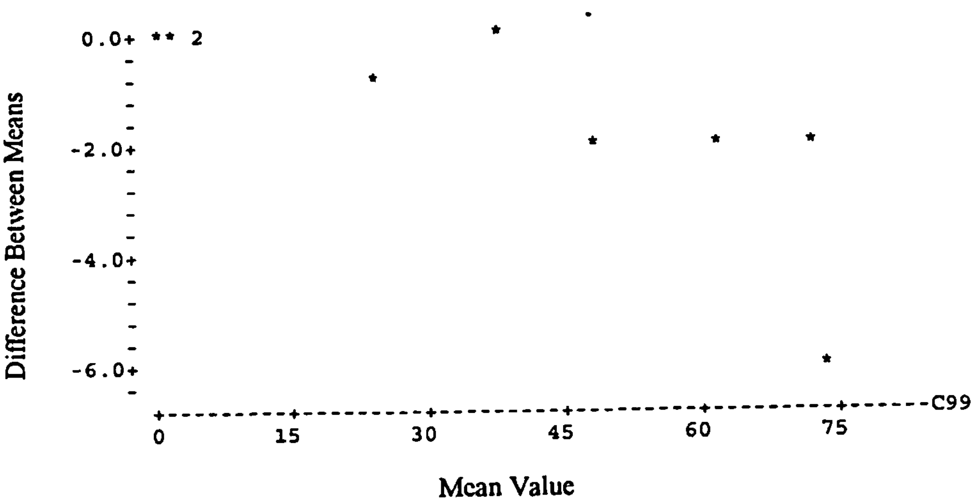


Figure 5.22: Scatterplot To Highlight Trends In Inter-Observer Error For Secondary Osteon Counts In Position 3

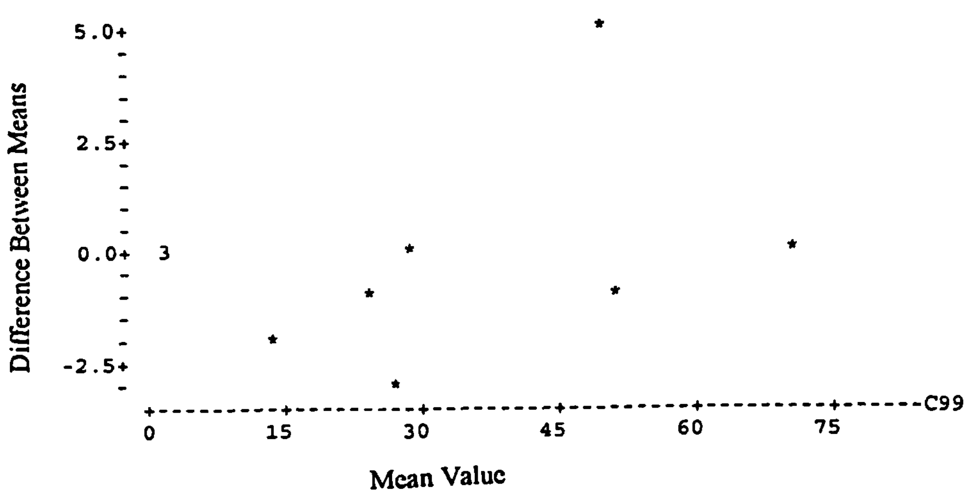


Figure 5.23: Scatterplot To Highlight Trends In Inter-Observer Error For Secondary Osteon Counts In Position 4

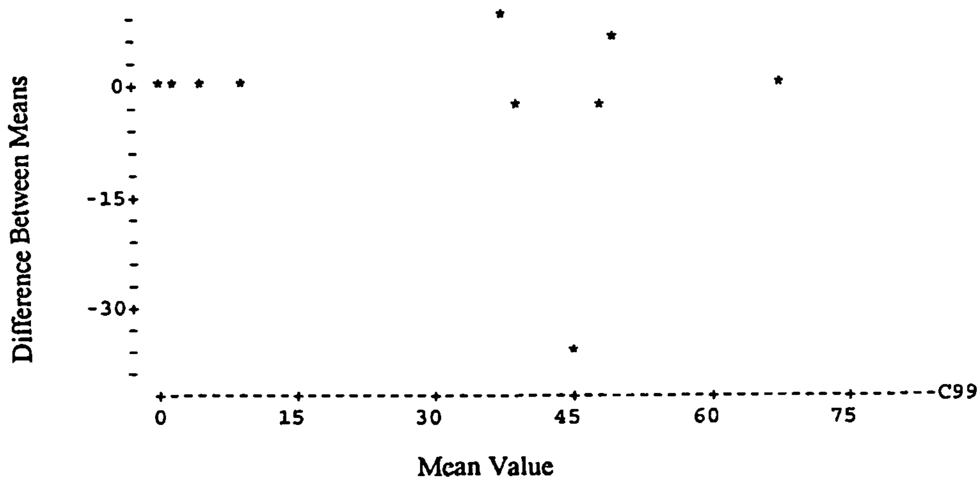


Figure 5.24: Scatterplot To Highlight Trends In Inter-Observer Error For Total Secondary Osteon Counts

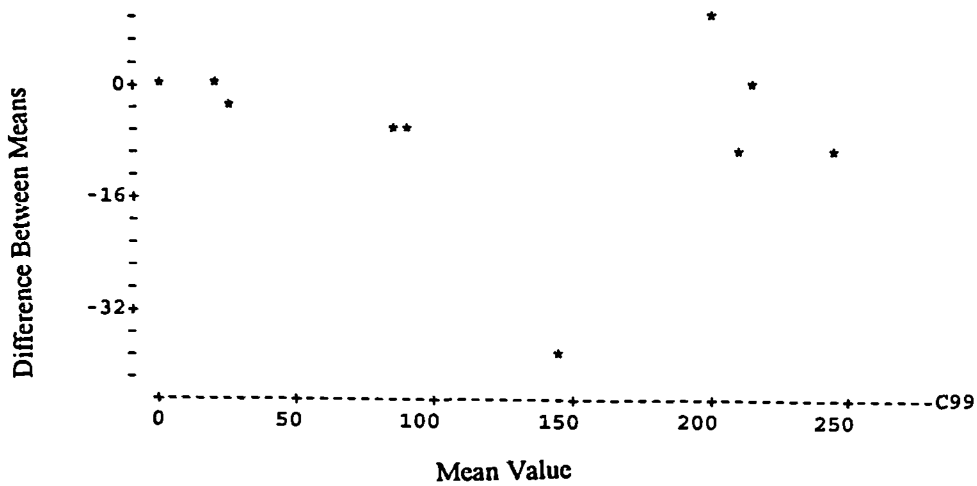


Figure 5.25: Scatterplot To Highlight Trends In Inter-Observer Error For Average Secondary Counts

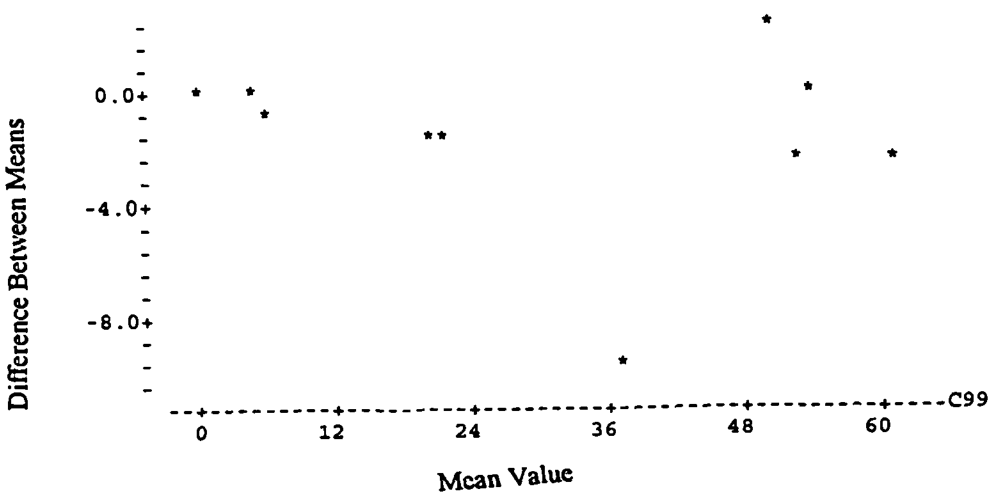


Figure 5.26: Scatterplot To Highlight Trends In Inter-Observer Error For Circumferential Lamellae Area In Position 1

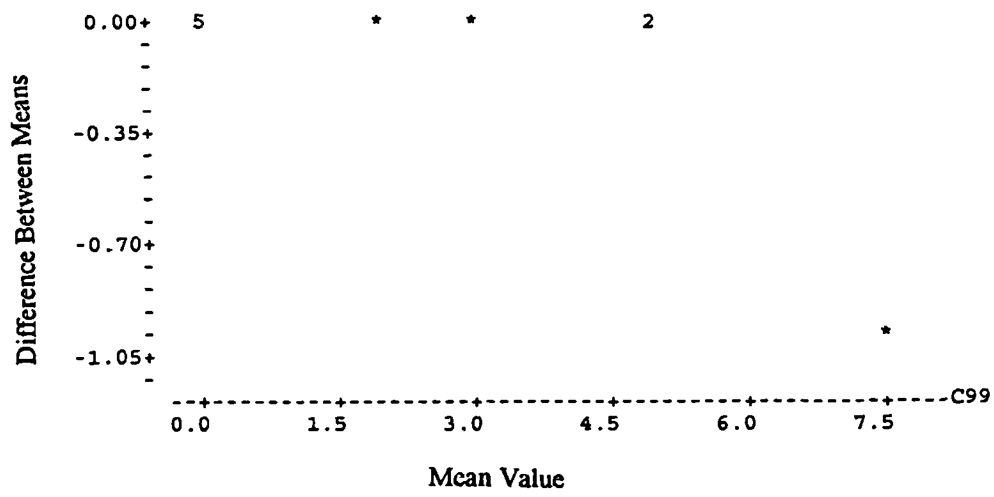


Figure 5.27: Scatterplot To Highlight Trends In Inter-Observer Error For Circumferential Lamellae Area In Position 2

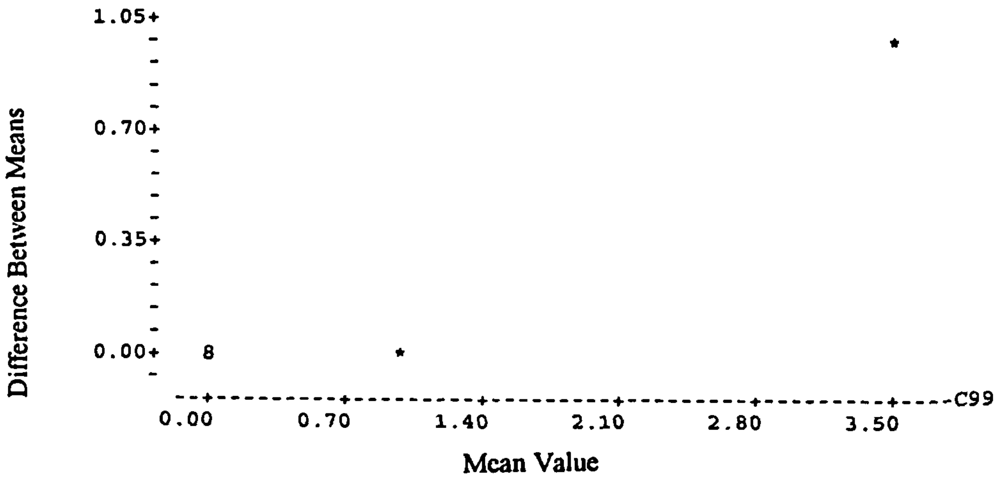


Figure 5.28: Scatterplot To Highlight Trends In Inter-Observer Error For Circumferential Lamellae Area In Position 3

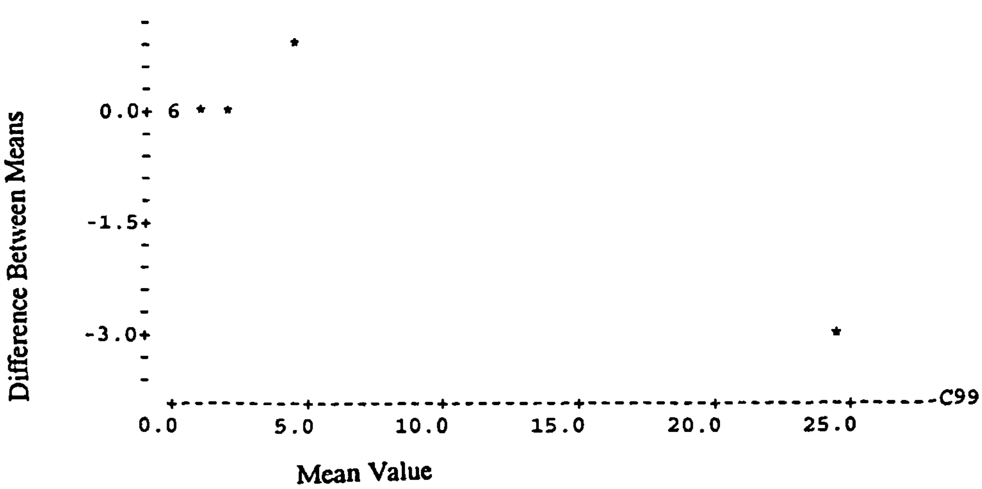


Figure 5.29: Scatterplot To Highlight Trends In Inter-Observer Error For Circumferential Lamellae Area In Position 4

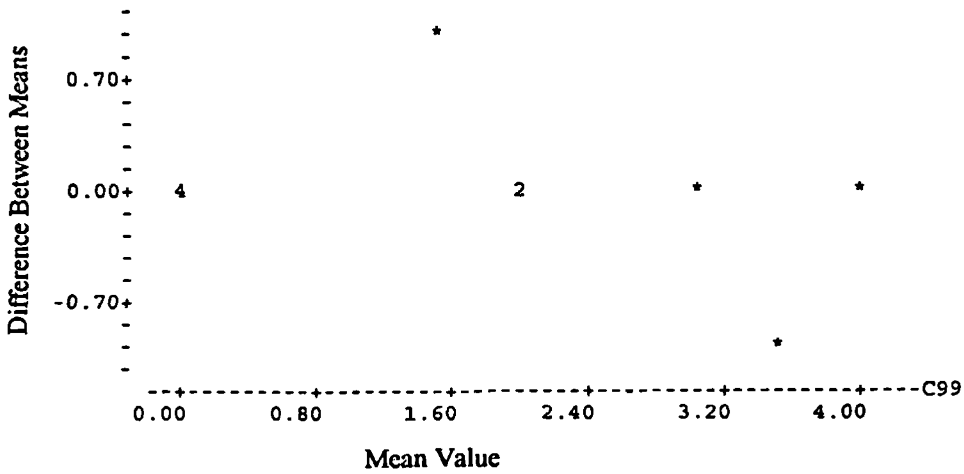


Figure 5.30: Scatterplot To Highlight Trends In Inter-Observer Error For Total Circumferential Lamellae Area

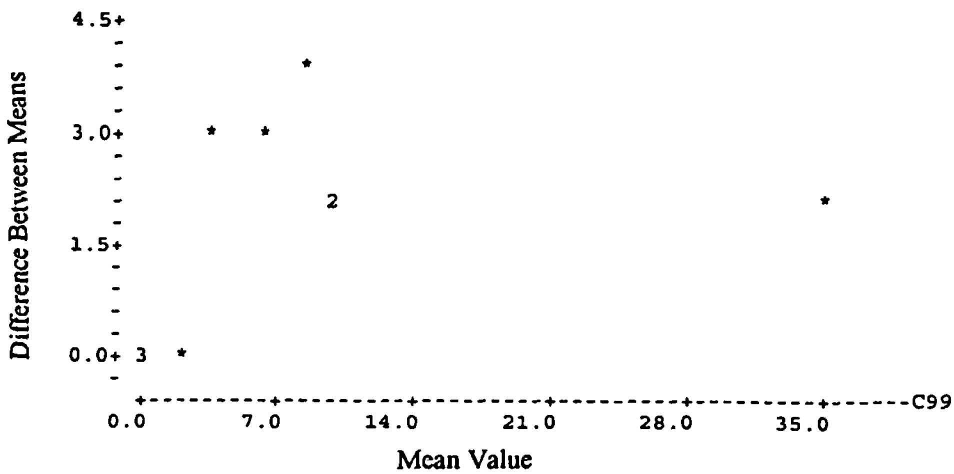


Figure 5.31: Scatterplot To Highlight Trends In Inter-Observer Error For Average Circumferential Lamellae Area

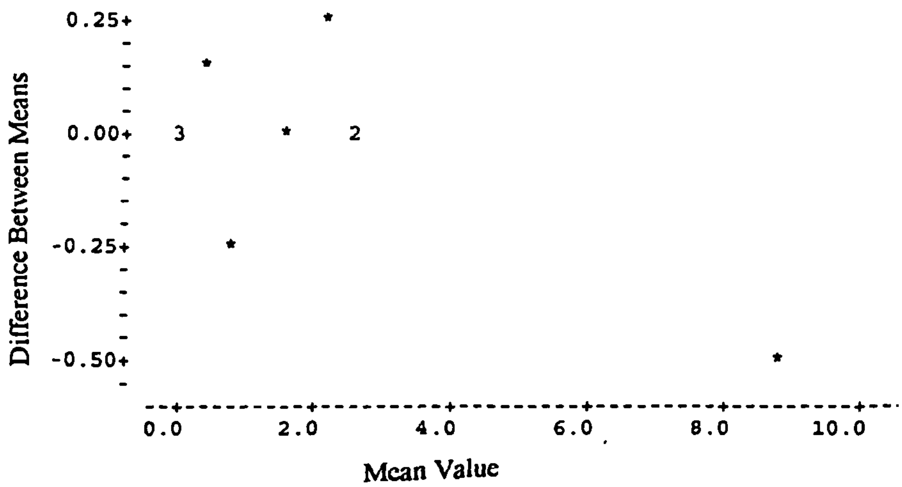


Figure 5.32: Scatterplot To Highlight Trends In Inter-Observer Error For Non-Haversian Canal Counts In Position 1

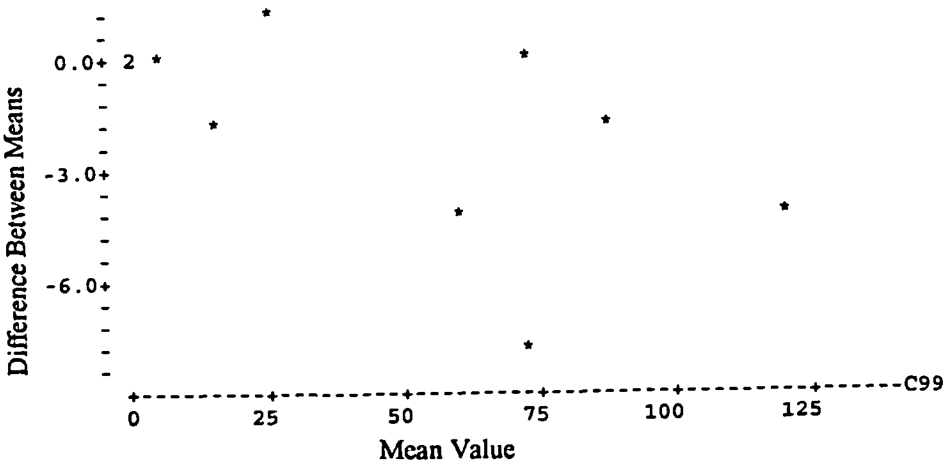


Figure 5.33: Scatterplot To Highlight Trends In Inter-Observer Error For Non-Haversian Canal Counts In Position 2

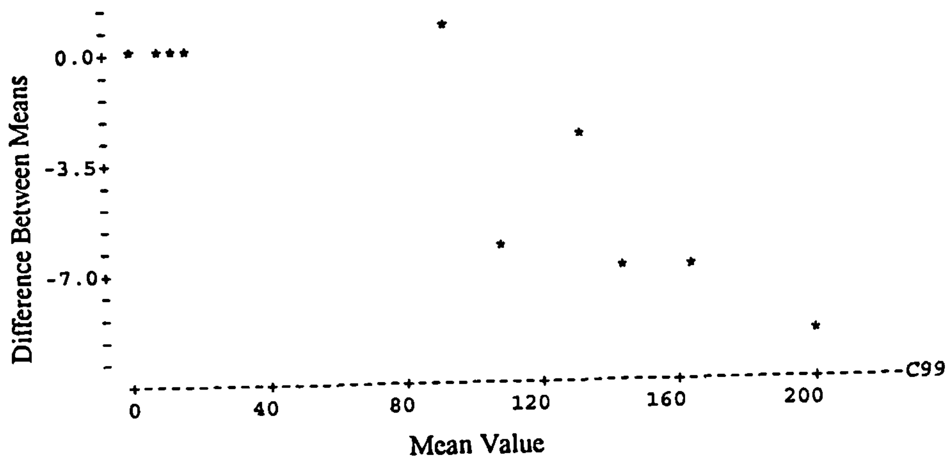


Figure 5.34: Scatterplot To Highlight Trends In Inter-Observer Error For Non-Haversian Canal Counts In Position 3

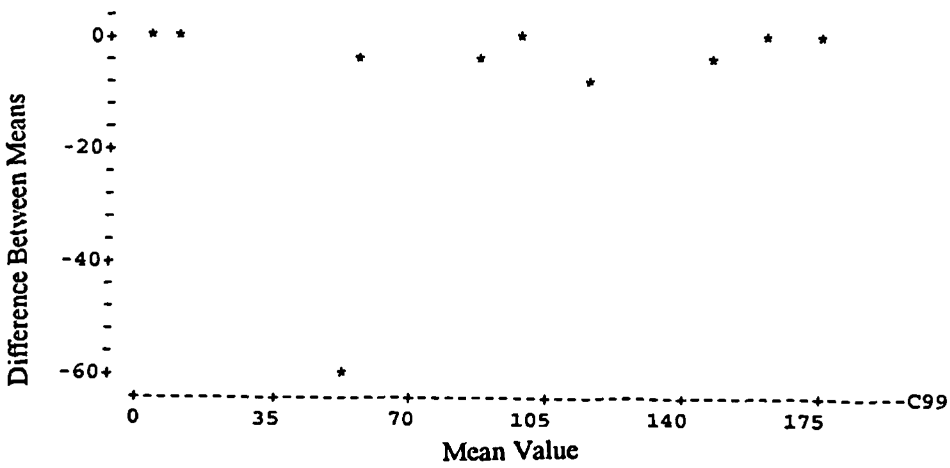


Figure 5.35: Scatterplot To Highlight Trends In Inter-Observer Error For Non-Haversian Canal Counts In Position 4

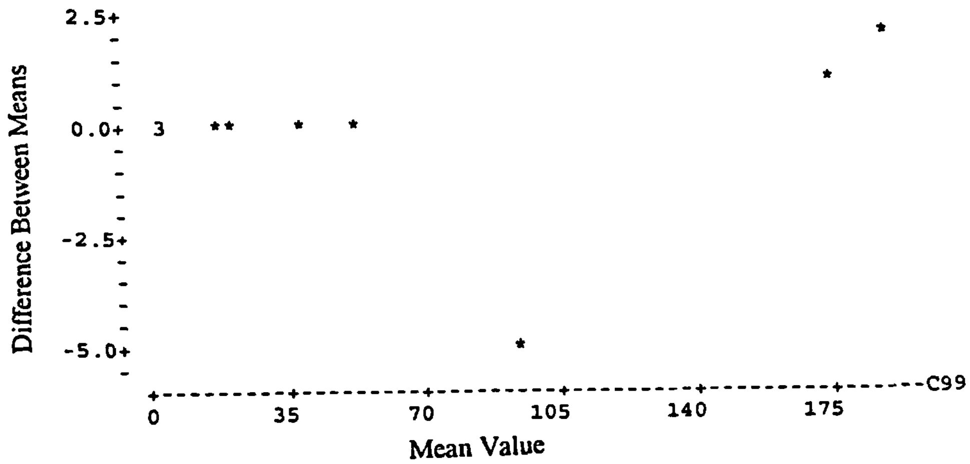


Figure 5.36: Scatterplot To Highlight Trends In Inter-Observer Error For Total Non-Haversian Canal Counts

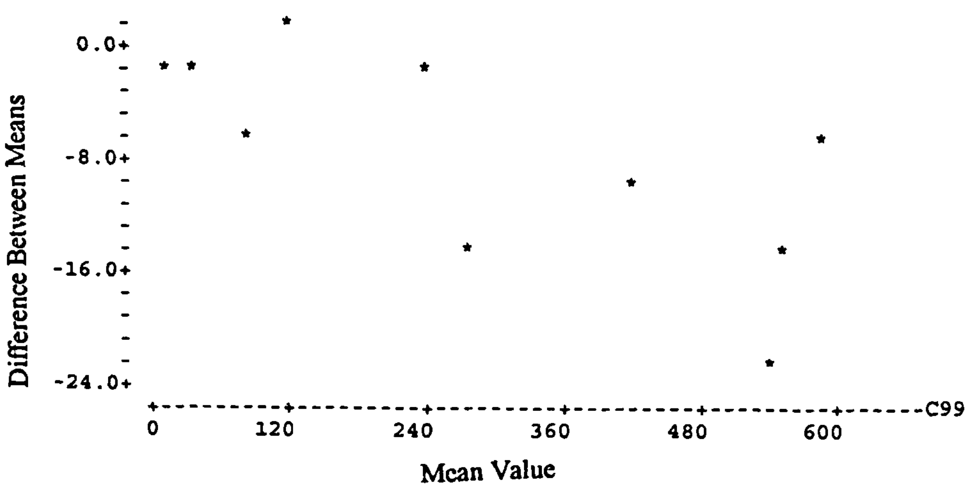
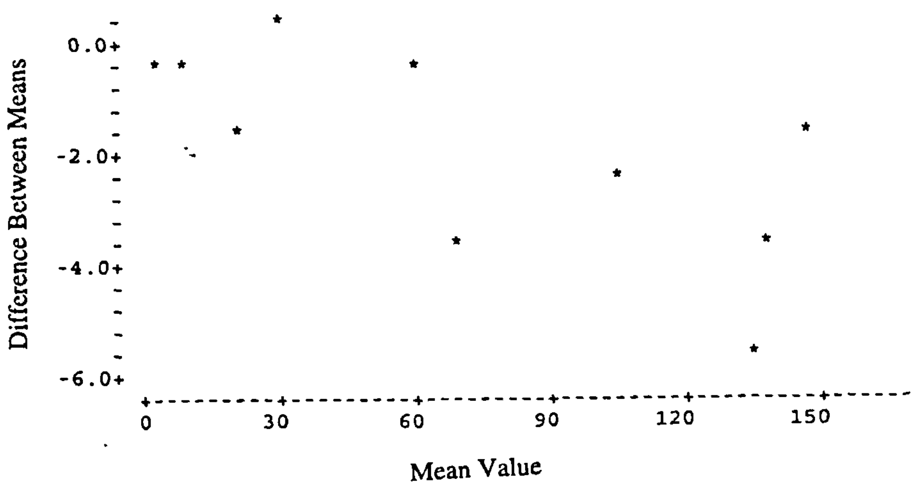


Figure 5.37: Scatterplot To Highlight Trends In Inter-Observer Error For Average Non-Haversian Canal Counts



Non-Haversian Canals

- A half of all paired counts showed differences.
- Some of observer B's counts were slightly larger than the original counts, but more tended to be smaller.
- There was no overall pattern or trend to the differences between counts.

5.1.2.3 In Summary

- t-tests showed that there were no significant differences between the paired counts of the original observer and observer B.
- The scatterplots showed that, although there were differences between some individual counts in each paired series, there were no specific inter-observer error trends and that features were not consistently under or over recorded during counting. However it was noted that, where a differences was present, observer B's counts were generally smaller than original counts.

5.1.3 IN CONCLUSION

The studies of intra- and inter-observer error showed that the adopted recording procedure was extremely reliable and, that, there were no significant differences or trends to differences between counts. Subsequently, all original counts were used as the basis for the statistical investigation of relationships between bone histology and cement layer age in roe deer.

5.2 Spearman's Rank Correlation Of Histological Counts And Cement Layer Ages

Spearman's rank correlation is a non-parametric test of association between variables. That is, it is not based on the assumption that there is a linear relationship between variables (Norušis, p.381). It was used to provide a preliminary insight into relationships between bone histology and cement layer age in roe deer. Spearman's rank correlation coefficients were computed for all histological counts and cement layer ages for:

5.2.1 The Whole Sample (Table 5.5)

Table 5.5: Spearman's Rank Correlation Of Secondary Osteon, Circumferential Lamellar Bone Area And Non-Haversian Canal Counts With Cement Layer Ages, For The Whole Sample (n=72)

Feature Count*	SRCC	*P*	Feature Count*	SRCC	*P*	Feature Count*	SRCC	*P*
OST1	0.52	0.00	CIRC1	0.02	0.89	N-HAV1	-0.64	0.00
OST2	0.47	0.00	CIRC2	0.17	0.16	N-HAV2	-0.58	0.00
OST3	0.58	0.00	CIRC3	0.19	0.11	N-HAV3	-0.63	0.00
OST4	0.48	0.00	CIRC4	0.00	0.98	N-HAV4	-0.56	0.00
ALLOST	0.55	0.00	ALLCIRC	0.10	0.41	ALLN-HAV	-0.68	0.00
AVEOST	0.55	0.00	AVECIRC	0.10	0.42	AVEN-HAV	-0.68	0.00

Table 5.6: Spearman's Rank Correlation Of Secondary Osteon, Circumferential Lamellar Bone Area And Non-Haversian Canal Counts With Cement Layer Ages, For The Bucks (n=30)

Feature Count*	SRCC	*P*	Feature Count*	SRCC	*P*	Feature Count*	SRCC	*P*
OST1	0.40	0.03	CIRC1	0.06	0.77	N-HAV1	-0.37	0.05
OST2	0.28	0.14	CIRC2	0.15	0.43	N-HAV2	-0.41	0.03
OST3	0.29	0.13	CIRC3	0.36	0.05	N-HAV3	-0.35	0.06
OST4	0.19	0.32	CIRC4	0.05	0.79	N-HAV4	-0.26	0.18
ALLOST	0.31	0.11	ALLCIRC	0.24	0.23	ALLN-HAV	-0.44	0.02
AVEOST	0.32	0.09	AVECIRC	0.24	0.23	AVEN-HAV	-0.44	0.02

Table 5.7: Spearman's Rank Correlation Of Secondary Osteon, Circumferential Lamellar Bone Area And Non-Haversian Canal Counts With Cement Layer Ages, For The Does (n=42)

Feature Count*	SRCC	*P*	Feature Count*	SRCC	*P*	Feature Count*	SRCC	*P*
OST1	0.50	0.00	CIRC1	0.02	0.89	N-HAV1	-0.70	0.00
OST2	0.50	0.00	CIRC2	0.13	0.43	N-HAV2	-0.58	0.00
OST3	0.66	0.00	CIRC3	0.06	0.72	N-HAV3	-0.70	0.00
OST4	0.60	0.00	CIRC4	-0.09	0.57	N-HAV4	-0.70	0.00
ALLOST	0.60	0.00	ALLCIRC	0.01	0.96	ALLN-HAV	-0.70	0.00
AVEOST	0.61	0.00	AVECIRC	0.00	0.98	AVEN-HAV	-0.74	0.00

5.2.2 The Bucks (Table 5.6)

5.3.3 The Does (Table 5.7)

5.2.1 The Whole Sample

Secondary Osteon Counts

- All counts showed highly significant (P=0.00) positive correlations with cement layer ages.
- Coefficients ranged from 0.47 for the position 2 (OST2) count to 0.58 for the position 3 count (OST3).

Circumferential Lamellar Bone Area

- Counts did not show any significant (P>0.10) correlations with cement layer ages.
- All coefficients fell between 0.00 and 0.19.

Non-Haversian Canal Counts

- These counts provided the strongest correlations with cement layer ages, for the whole sample.
- All coefficients were highly significant (P=0.00) and negative, and they ranged from -0.56 for the position 4 count (N-HAV4) to -0.68 for both the total and average counts (ALLN-HAV and AVEN-HAV).

5.2.2 The Bucks

Secondary Osteon Counts

- All counts showed positive correlations with cement layer ages, but the degrees of association were not as strong or as significant as they were for the whole sample.
- Coefficients ranged from 0.19 (P=0.32) for the position 4 count (OST4) to 0.40 (P=0.03) for the position 1 count (OST1).

Circumferential Lamellar Bone Area

- All coefficients were close to 0.00 and ranged from 0.05 (*P*=0.79) for the position 4 count (CIRC4) to 0.36 (*P*=0.05) for the position 3 count (CIRC3).

Non-Haversian Canal Counts

- These showed stronger correlations with cement layer ages than secondary osteon counts and circumferential lamellar bone areas.
- Coefficients were negative, but they were not as strong or as significant as for the whole sample. They ranged from -0.26 (*P*=0.18) for the position 4 count (N-HAV4) to -0.44 (*P*=0.02) for the total and average counts (ALLN-HAV and AVEN-HAV).

5.2.3 The Does

Secondary Osteon Counts

- These counts showed stronger correlations with cement layer ages, than similar counts for the whole sample or the bucks.
- All coefficients were highly significant and ranged from 0.50 (*P*=0.00) for the position 1 count (OST1) to 0.66 (*P*=0.00) for the position 3 count (OST3).

Circumferential Lamellar Bone Area

- These counts did not show any significant correlations with cement layer ages.
- All coefficients were below 0.15 (*P*>0.40).

Non-Haversian Canal Counts

- These counts showed the strongest and most highly significant correlations with cement layer ages of all feature counts.
- All coefficients were negative and ranged from -0.58 (*P*=0.00) for the position 2 count (N-HAV2) to -0.74 (*P*=0.00) for the average count (AVEN-HAV).

5.2.4 IN SUMMARY

- Secondary osteon counts showed highly significant positive correlations with cement layer ages, for the whole sample and for the doe sample. This indicated that secondary osteon count increased as cement layer age increased.
- Circumferential lamellar bone area counts did not show any significant correlations with cement layer ages, for the sample as a whole and when the sexes were analysed separately.
- Non-Haversian canal counts showed the strongest correlations with cement layer ages. All coefficients were negative and most were highly significant ($P<0.05$). This indicated that non-Haversian canal count decreased as cement layer age increased. A stronger degree of association was noted between non-Haversian canal counts and cement layer ages in the doe sample than the buck sample.

5.2.5 DISCUSSION

As in humans (Chapter 3.1), there was a degree of positive association between age (here cement layer age) and secondary osteon count, and negative association between age and non-Haversian canal count. Like Ericksen (1991; Table 3.9), the degree of association between age and bone histology feature was stronger for the females (does) than the males (bucks). Here this may be due to:

- The stresses placed on the bucks during the production of antlers each year. Roe antlers grow during winter, when food is scarce, and it is likely that the huge demands placed on the body to produce successive sets of antlers disrupt other normal growth procedures, such as bone turnover.
- Similarly, during the rut, which takes place from late June to early August, roe bucks starve themselves and this, coupled with the huge hormonal imbalances occurring at this time, is likely to disrupt growth and bone turnover.

It is noted that in does pregnancy may also disrupt growth and bone turnover. This may be why, although there was a significant degree of association between age and secondary osteon and non-Haversian canal counts, it was not a consistently strong degree of association - coefficients ranged from 0.50 for secondary osteon count in position 1 to -0.74 for average non-Haversian canal count.

In contrast to humans, no significant strong association between age and circumferential lamellar bone area was noted for either bucks or does. This may be because:

- *Circumferential lamellae had been stripped away from the outer surface of the bone during* preparation (Chapter 4.2). An effort was made to exclude specimens where this was apparent.
- The cement layer ages given were incorrect. This seems highly unlikely and, although their validity cannot be known, the consistency of relationships between them and histological feature counts, and between them and tooth wear scores (Chapter 5.6), suggests that they are representative of the true ages of the sample, or at the very least a consistent measure of the range of ages of the sample material.
- In the area of the mandible studied, there was no association between cement layer age and circumferential lamellar bone. There is nothing to suggest that this is not the true case.

5.2.6 In Conclusion

- There was some degree of association between secondary osteon and non-Haversian canal counts in roe deer, and that degree of association was stronger for does than bucks.
- There was no association between circumferential lamellar bone area and cement layer age, and this feature was given no further consideration in this study.

5.3 Regression Of Histological Feature Counts Against Cement Layer Ages

Linear regression models (Norušis, p.389) were fitted to the relationships between all secondary osteon and non-Haversian canal counts and cement layer ages, for:

5.3.1 The Whole Sample

5.3.2 The Bucks

5.3.3 The Does

The models were fitted with cement layer age as the independent variable (x) and histological count as the dependent variable (y). Studentised deleted residuals were analysed for each model to confirm that each histological count adhered to the assumptions necessary for the application of linear regression (Noruŝis, pp.447-464): The four assumptions of linear regression are:

1. Normal distribution of the dependent variable.
2. Constant variance of the dependent variable.
3. Linear relationship between independent and dependent variables.
4. Independence, from each other, of the dependent variables

Some counts (below) did not adhere to the assumptions of linear regression, and so it was necessary to transform the count using a square root transformation (Noruŝis, p. 458). A new linear regression model was then fitted to the relationship between transformed histological count and cement layer age. For each, appropriately fitted, regression model a table is presented which details:

- The slope and intercept of the regression line (Noruŝis, p. 393).
- The Pearson's correlation coefficient: This indicates the strength of the linear association between the histological count and cement layer age (Noruŝis, p. 400).
- r^2—This the Pearson's correlation coefficient squared, and it indicates what proportion of a change in histological count can be accounted for by a change in cement layer age (Noruŝis, p. 403).
- The K-S (Lilliefors) significance: This is a measure of normality and was used to assess whether or not values within the original histological count were normally distributed (Noruŝis, p. 453). For normal distribution its value should be >0.05.
- The Durbin-Watson statistic: This is a measure of independence and was used to assess whether or not values within the original histological count were obtained independently. To confirm independence its value should be between 1.5 and 2.5 (Noruŝis, p. 460).

Three figures are also presented for each, appropriately fitted, regression model:

1. A Q-Q plot of studentised deleted residuals: This also allows normality to be assessed. It shows the plot of the actual observed studentised deleted residuals against those expected for a normally distributed count. Normal distribution is indicated when the majority of points in the plot fall on close to a straight line (Noruŝis, p. 452).
2. A scatterplot of studentised deleted residuals against the predicted value of histological count generated through the linear regression model: This allows both constant variance and linearity

to be assessed. If variance in histological count is constant for each cement layer age, and there is a linear relationship between the two variables then the points within the scatterplot should be randomly distributed around 0 (Norušis, pp. 454-457).

3. The fitted regression line with the 95% confidence intervals.

5.3.1 THE WHOLE SAMPLE

Regression of secondary osteon count in position 1 on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.8) and the majority of points in the Q-Q plot (Figure 5.38) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.39). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.77 (Table 5.8). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.48 (P=0.00) (Table 5.8). This indicated that there was a highly significant, weak linear relationship between the two variables.
- The value of r^2 was 0.23 (Table 5.8). This indicated that the regression model did not fit the data well and that only 23% of a change in secondary osteon count in position 1 could be accounted for by a change in cement layer age.

Table 5.8: Regression Of Secondary Osteon Count In Position 1 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
3.40	14.08	0.48 (P=0.00)	0.23	>0.20	1.77

Figure 5.38: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

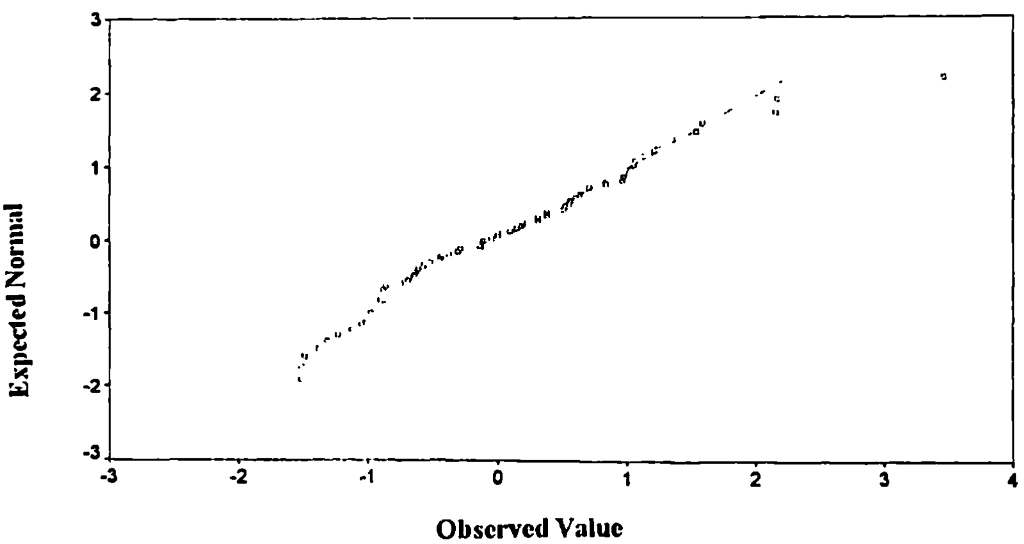


Figure 5.39: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

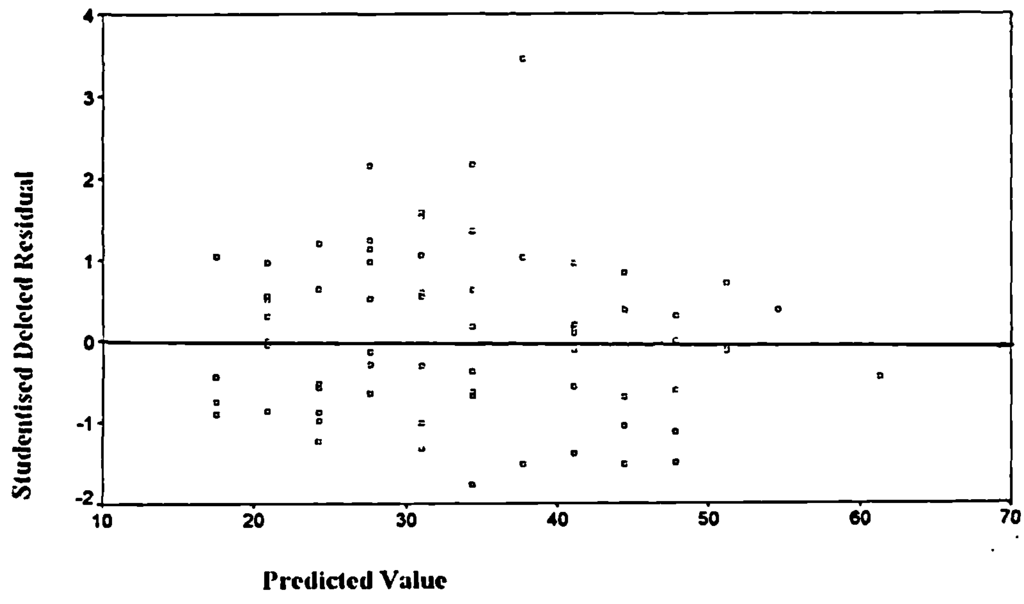
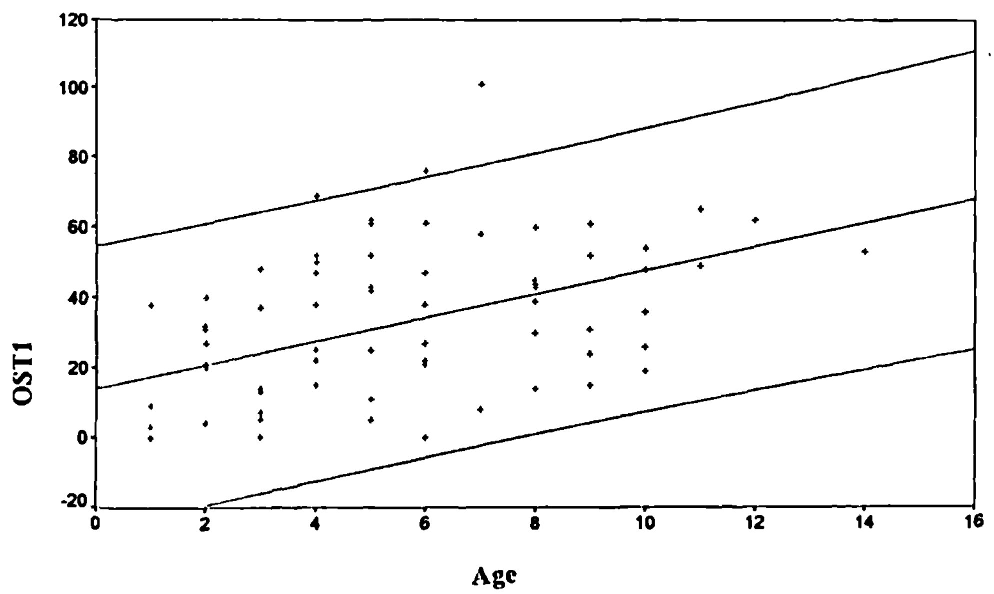


Figure 5.40: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 1 (OST1) And Cement Layer Age



Regression of secondary osteon count in position 2 on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.00. This indicated that the studentised deleted residuals were not normally distributed and that it was not appropriate to fit a linear regression model to the relationship between secondary osteon count in position 2 and cement layer age. Instead, values within the count were transformed and a new linear regression model was fitted to the relationship between the transformed secondary osteon count in position 2 and cement layer age (below).

Regression of transformed secondary osteon count in position 2 on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.9) and the majority of points in the Q-Q plot (Figure 5.41) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.42). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.61 (Table 5.9). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.50 (P=0.00) (Table 5.9). This indicated that there was a highly significant, weak linear relationship between the two variables.
- The value of r^2 was 0.25 (Table 5.9). This indicated that the regression model did not fit the data well and that only 25% of a change in transformed secondary osteon count in position 2 could be accounted for by a change in cement layer age.

Table 5.9: Regression Of Transformed Secondary Osteon Count In Position 2 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
0.49	2.71	0.50 (P=0.00)	0.25	>0.20	1.61

Figure 5.41: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

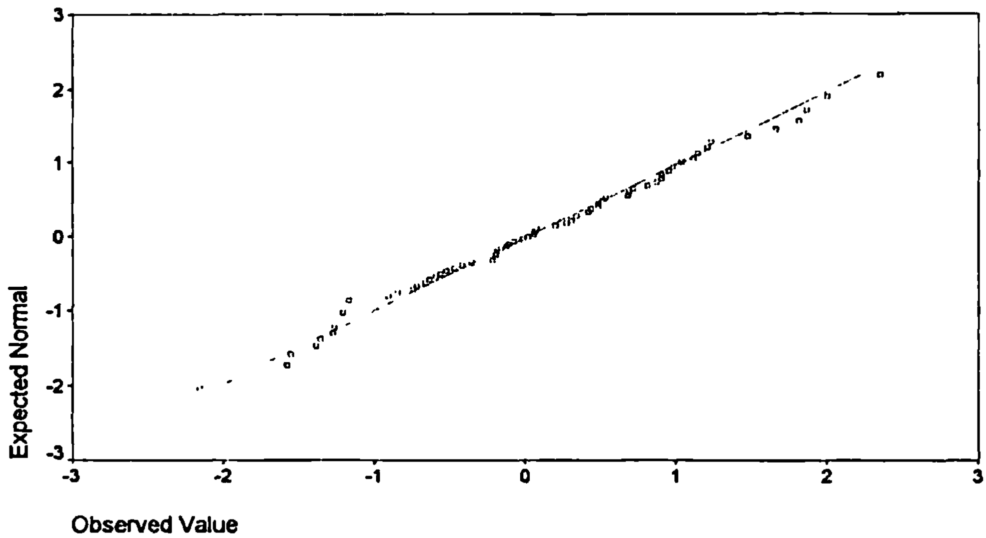


Figure 5.42: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

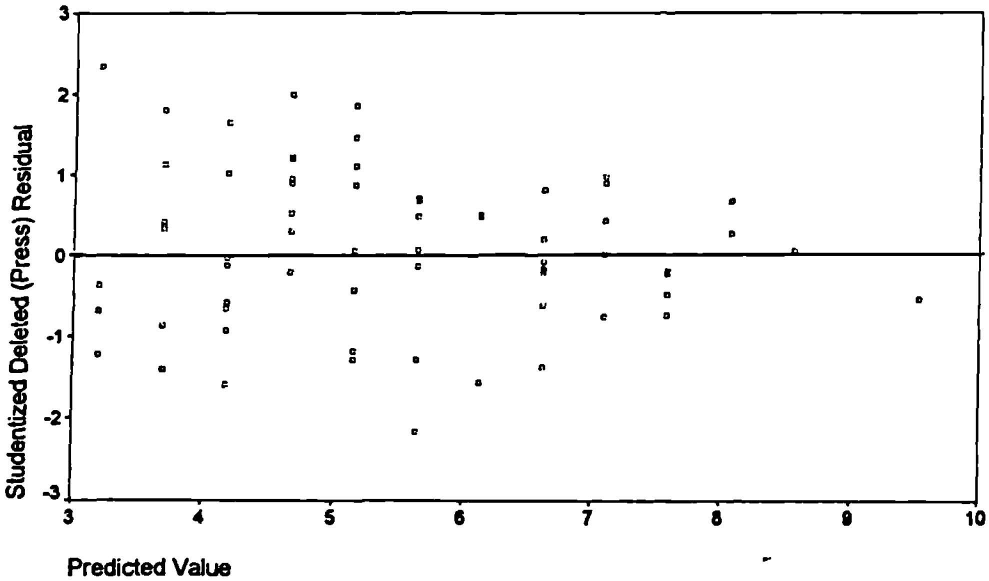
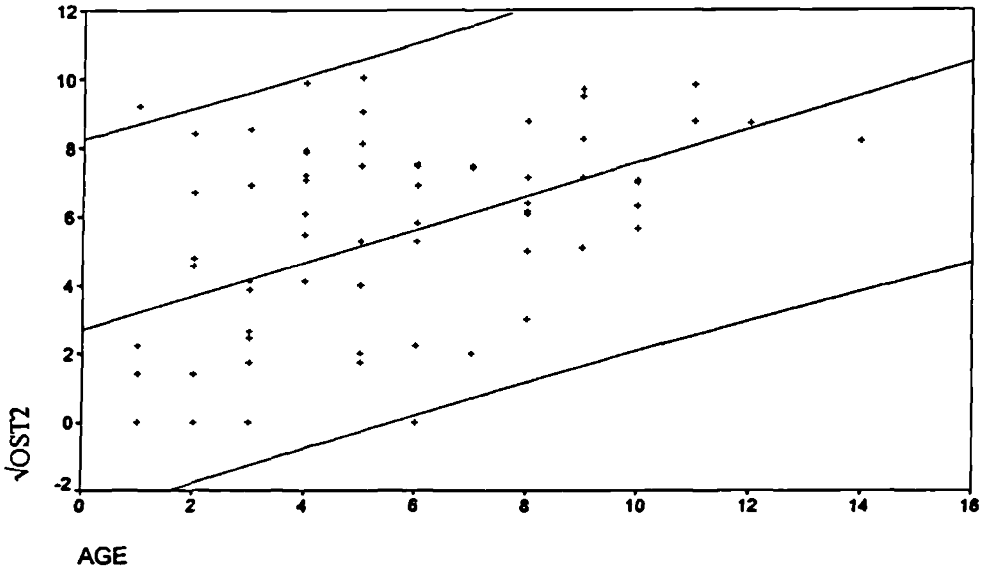


Figure 5.43: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Secondary Osteon Count In Position 2 (√OST2) And Cement Layer Age



Regression of secondary osteon count in position 3 on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.00. This indicated that the studentised deleted residuals were not normally distributed and, that, it was not appropriate to fit a linear regression model to the relationship between the two variables. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed secondary osteon count in position 3 and cement layer age (below).

Regression of transformed secondary osteon count in position 3 on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.10) and the majority of points in the Q-Q plot (Figure 5.44) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.45). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.56 (Table 5.10). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.57 (P=0.00) (Table 5.10). This indicated that there was a highly significant, weak linear relationship between the two variables.
- The value of r^2 was 0.32 (Table 5.10). This indicated that the regression model did not fit the data well and, that, only 32% of a change in transformed secondary osteon count in position 3 could be accounted for by a change in cement layer age.

Table 5.10: Regression Of Transformed Secondary Osteon Count In Position 3 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
0.53	1.44	0.57 (P=0.00)	0.32	>0.20	1.56

Figure 5.44: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

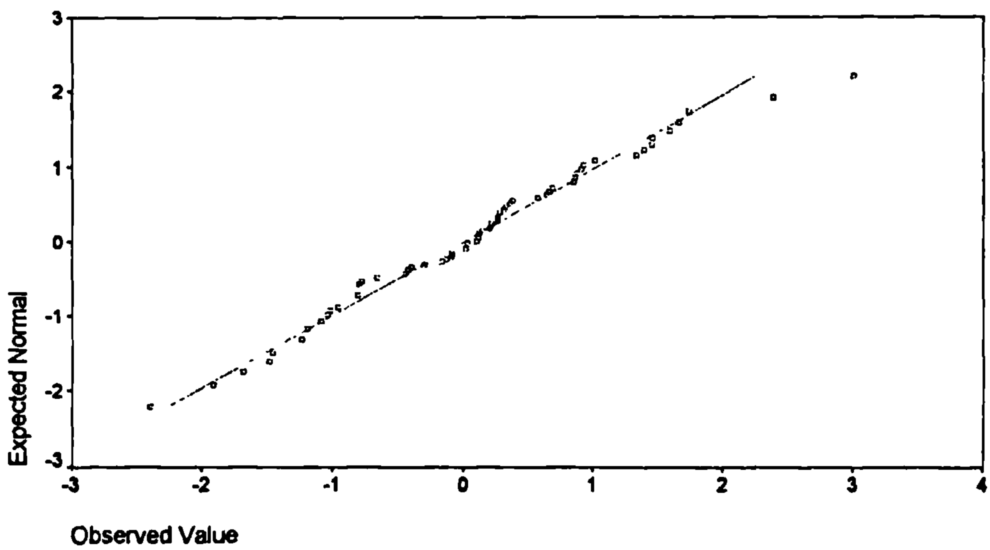


Figure 5.45: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

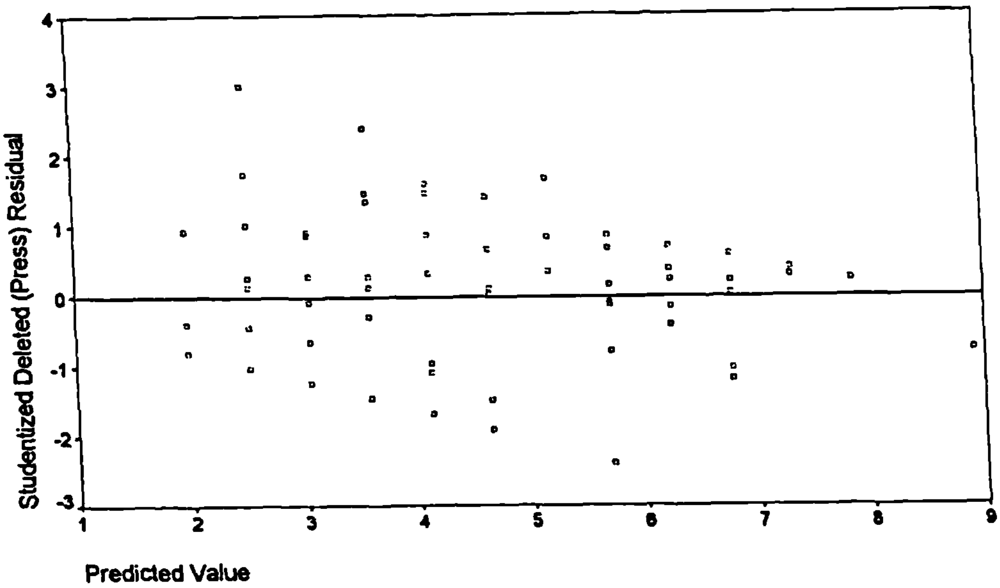
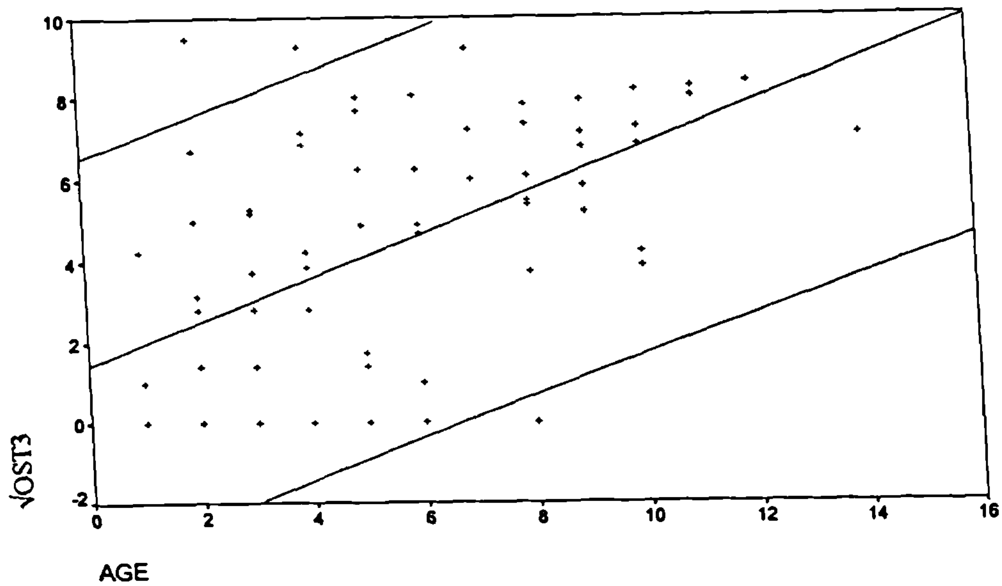


Figure 5.46: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Secondary Osteon Count In Position 3 (√OST3) And Cement Layer Age



Regression of secondary osteon count in position 4 on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.04 (Table 5.11) and the majority of points in the Q-Q plot (Figure 5.47) fell around a straight line. The K-S (Lilliefors) significance was on the borderline of acceptability for normal distribution but, as the majority of points in the Q-Q plot did fall around a straight line and the count adhered to all other regression assumptions (below), it was not rejected, and a linear regression model was fitted.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.48). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.02 (Table 5.11). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.47 (P=0.00) (Table 5.11). This indicated that there was a highly significant weak linear relationship between the two variables.
- The value of r^2 was 0.22 (Table 5.11). This indicated that the regression model did not fit the data well and, that, only 22% of a change in secondary osteon count in position 4 could be accounted for by a change in cement layer age.

Table 5.11: Regression Of Secondary Osteon Count In Position 4 On Cement Layer Age

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
3.38	16.06	0.47 (P=0.00)	0.22	0.04	2.02

Figure 5.47: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

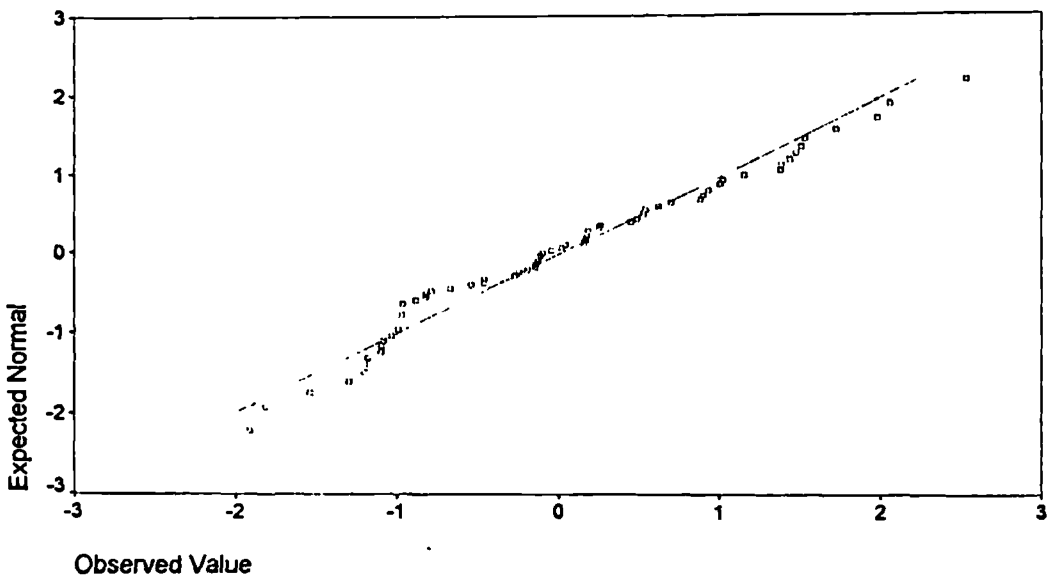


Figure 5.48: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

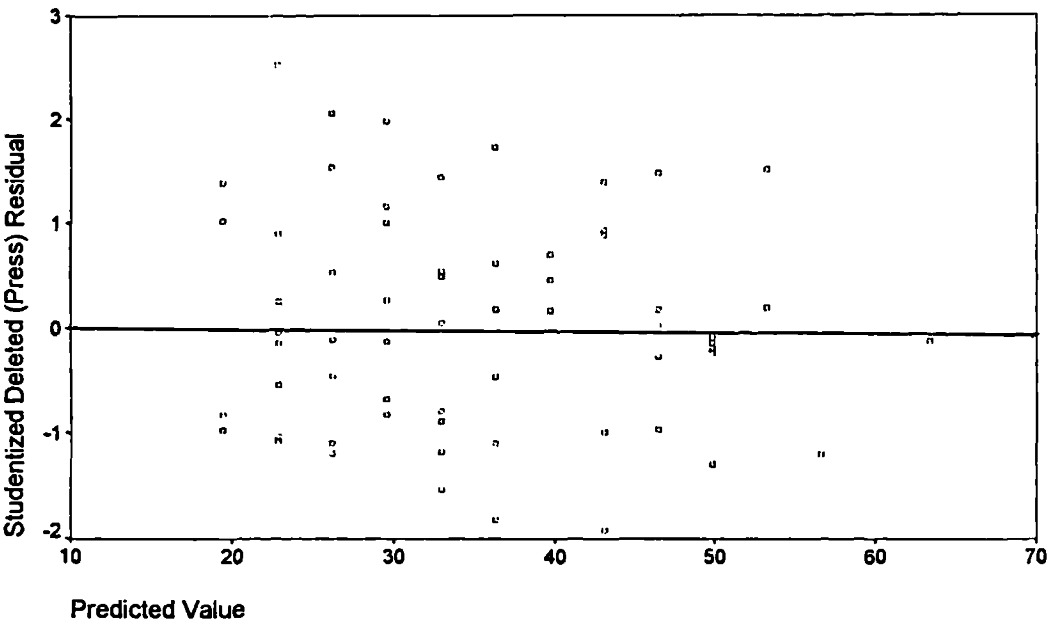
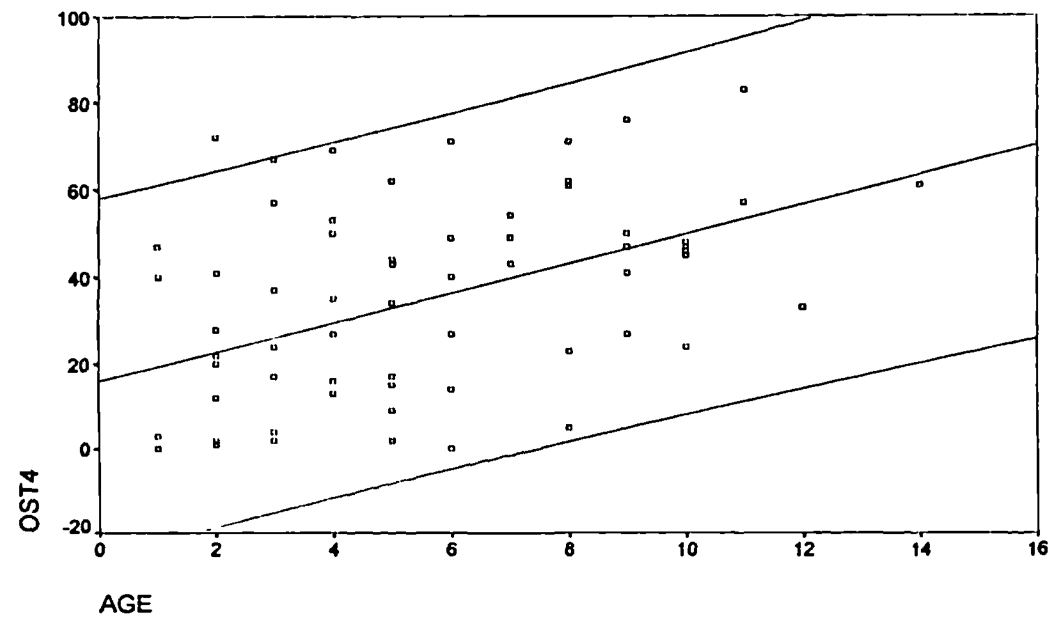


Figure 5.49: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 4 (OST4) And Cement Layer Age



Regression of total secondary osteon count on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.03. This indicated that the studentised deleted residuals were not normally distributed and, that, it was not appropriate to fit a linear regression model to the relationship between total secondary osteon count and cement layer age. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed total secondary osteon count and cement layer age (below).

Regression of *transformed* total secondary osteon count on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.2) and most of the points in the Q-Q plot (Figure 5.50) fell on, or close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.51). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.71 (Table 5.12). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.56 (P=0.00) (Table 5.12). This indicated that there was a highly significant weak linear relationship between the two variables.
- The value of r^2 was 0.32 (Table 5.12). This indicated that the linear regression model did not fit the data well, and that only 32% of a change in transformed total secondary osteon count could be accounted for by a change in cement layer age.

Table 5.12: Regression Of Transformed Total Secondary Osteon Count On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
0.87	5.72	0.56 (P=0.00)	0.32	>0.20	1.71

Figure 5.50: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

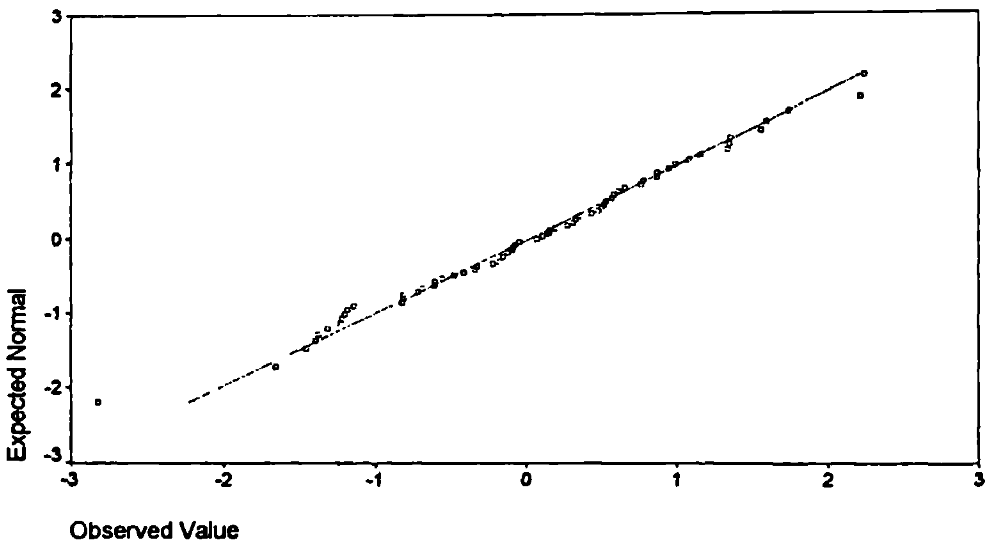


Figure 5.51: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

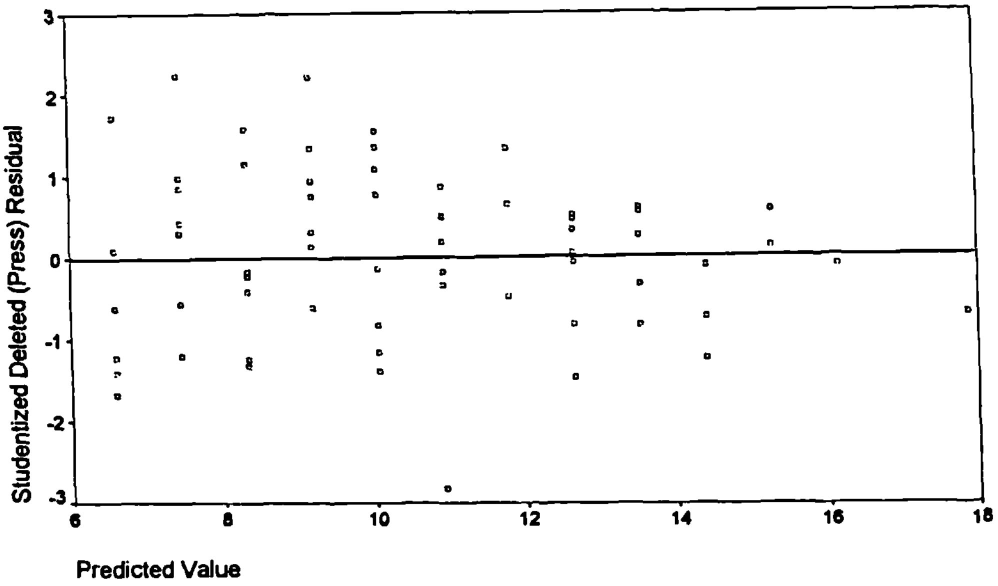
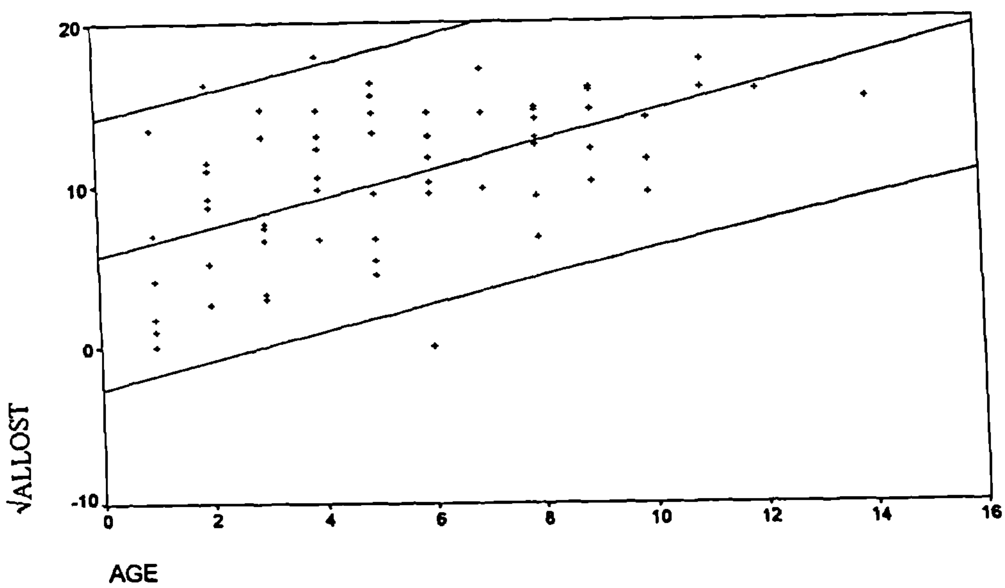


Figure 5.52: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Total Secondary Osteon Count (√ALLOST) And Cement Layer Age



Regression of average secondary osteon count on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.06 (Table 5.13) and most of the points in the Q-Q plot (Figure 5.53) fell on, or close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.54). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.72 (Table 5.13). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.55 (P=0.00) (Table 5.13). This indicated that there was a highly significant weak linear relationship between the two variables.
- The value of r^2 was 0.30 (Table 5.13). This indicated that the regression model did not fit the data well and, that, only 30% of a change in average secondary osteon count could be accounted for by a change in cement layer age.

Table 5.13: Regression Of Average Secondary Osteon Count On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
3.91	12.25	0.55 (P=0.00)	0.30	0.06	1.72

Figure 5.53: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

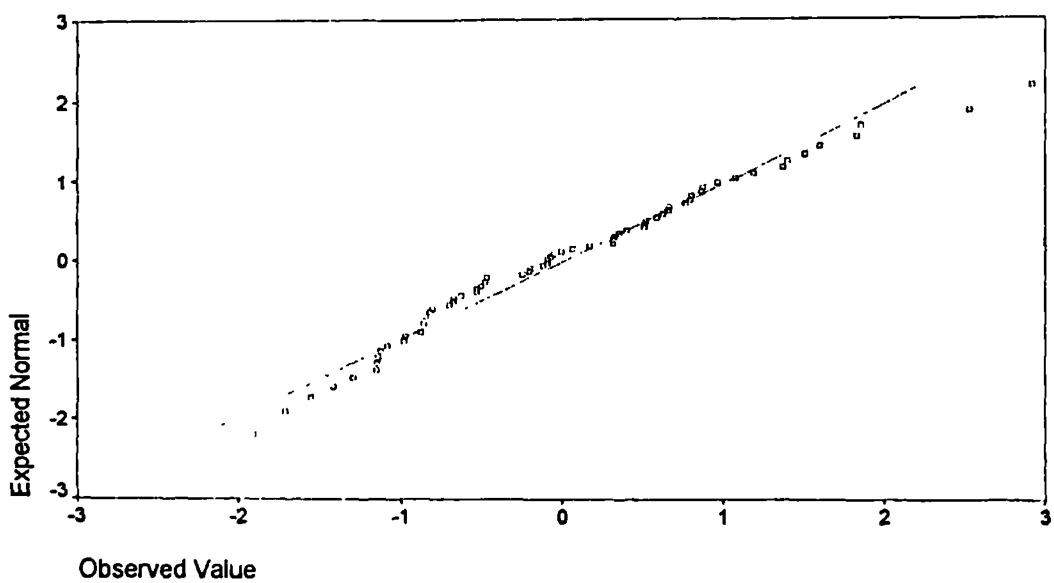


Figure 5.54: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

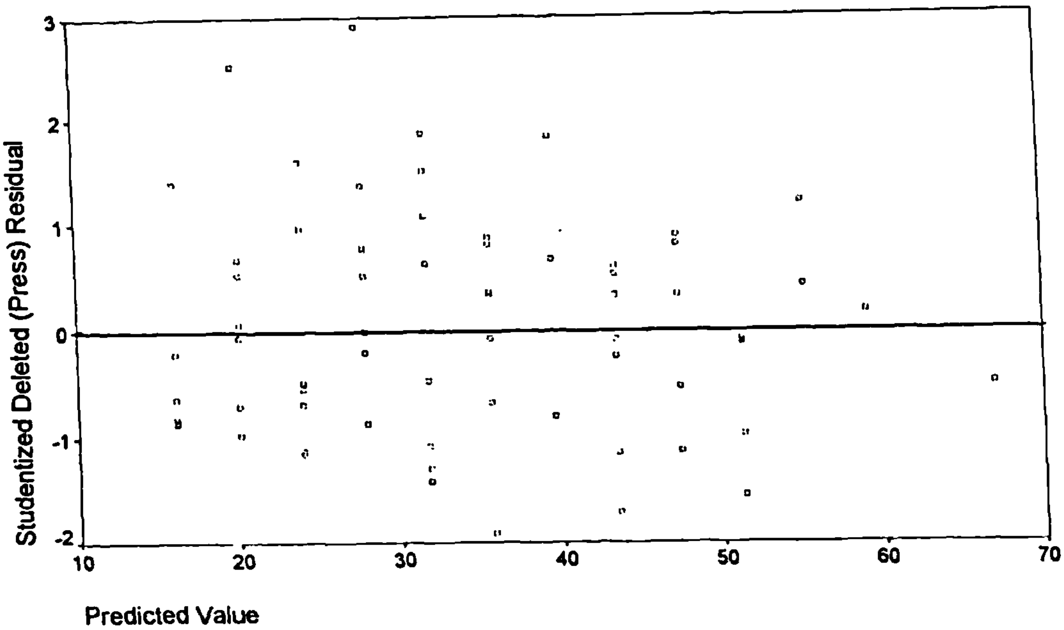
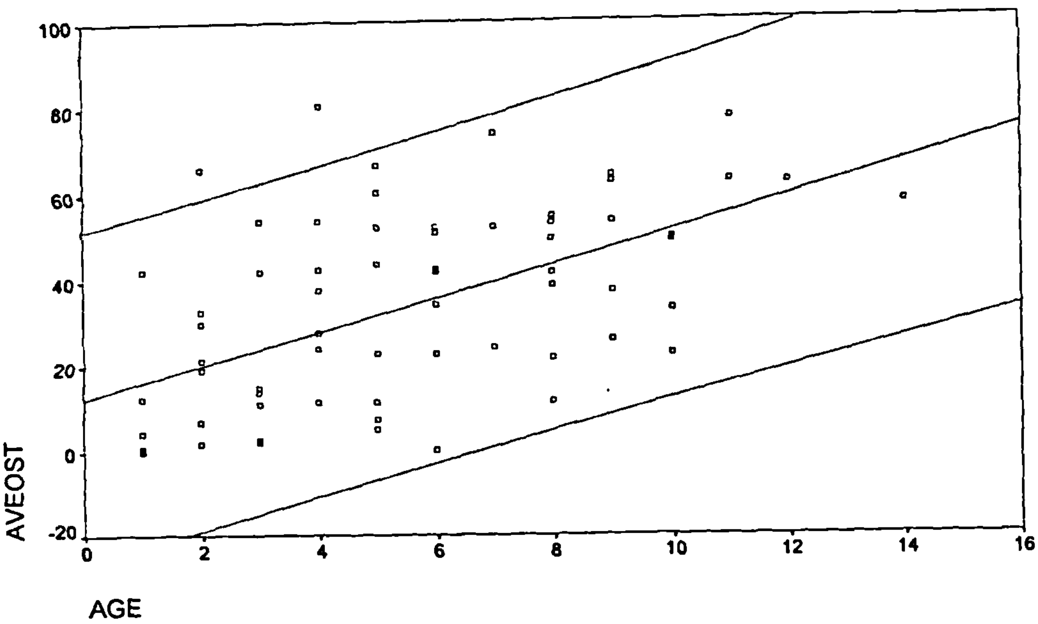


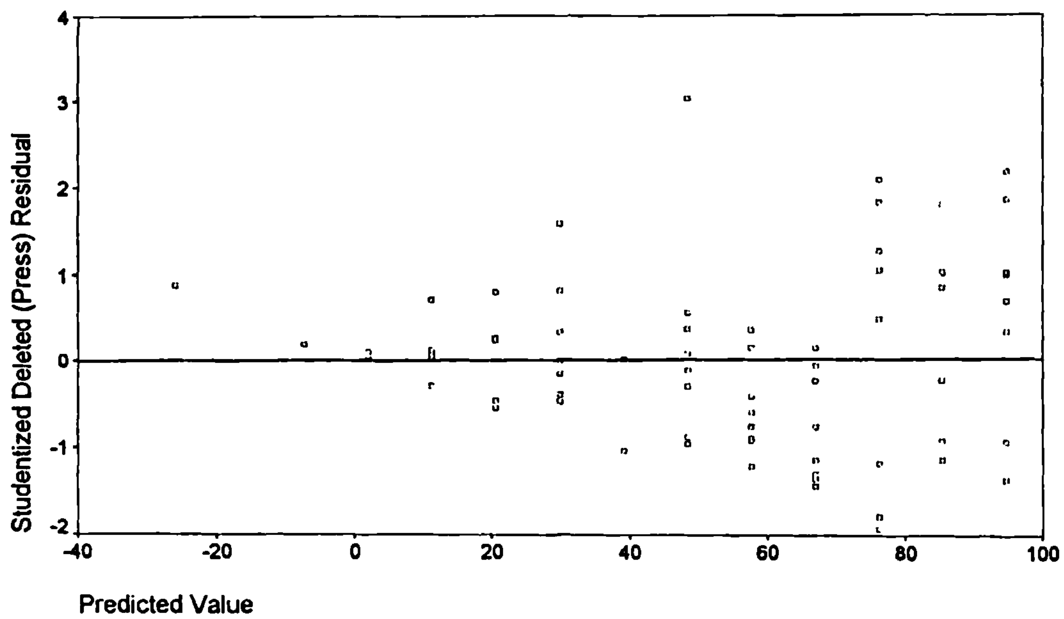
Figure 5.55: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Average Secondary Osteon Count (AVEOST) And Cement Layer Age



Regression of non-Haversian canal count in position 1 on cement layer age (n=72):

- The studentised deleted residuals were distributed around 0 (Figure 5.56) but, their distribution was not random, and their variability increased with an increase in predicted count. This indicated that the they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed non-Haversian canal count in position 1 and cement layer age (below).

Figure 5.56: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 1 and cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.14) and most points in the Q-Q plot (Figure 5.57) were distributed on, or very close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.58). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.89 (Table 5.14). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.65 (P=0.00) (Table 5.14). This indicated that there was a highly significant, moderately strong degree of linear association between transformed non-Haversian canal count in position 1 and cement layer age.
- The value of r^2 was 0.42 (Table 5.14). This indicated that the model fitted the data quite well and, that, 42% of a change in transformed non-Haversian canal count in position 1 could be accounted for by a change in cement layer age.

Table 5.14: Regression Of Transformed Non-Haversian Canal Count In Position 1 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.70	10.31	-0.65 (P=0.00)	0.42	>0.20	1.89

Figure 5.57: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

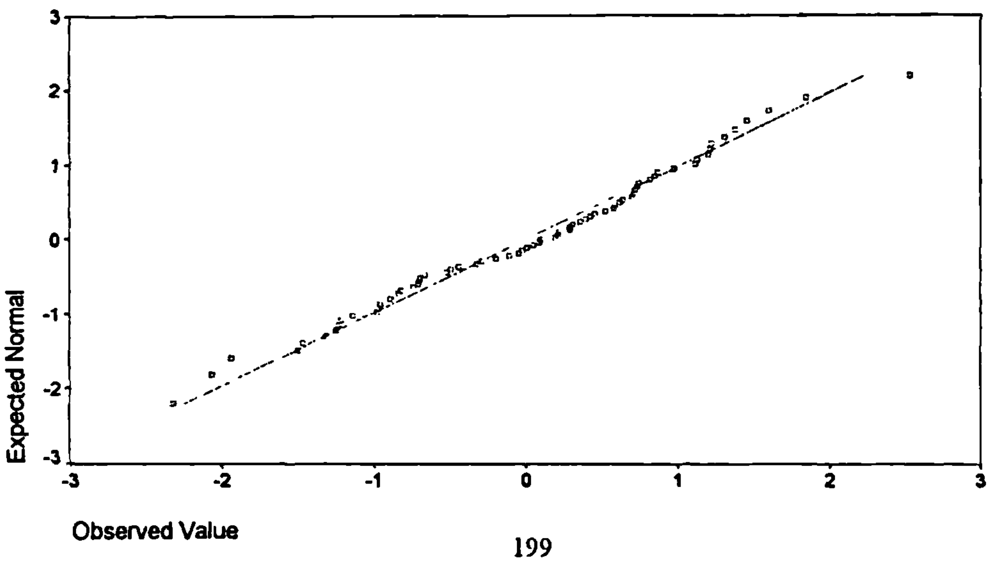


Figure 5.58: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

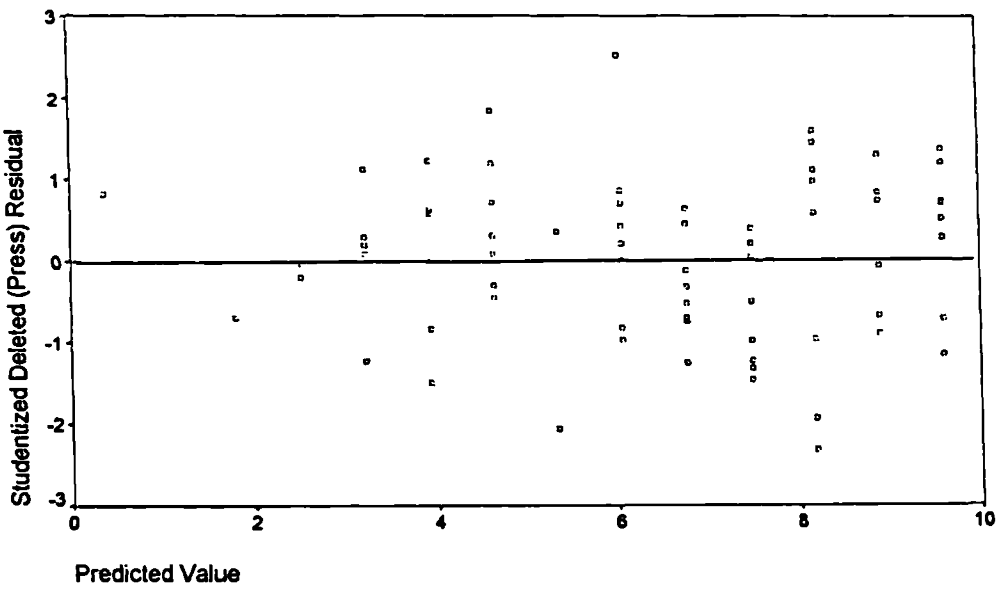
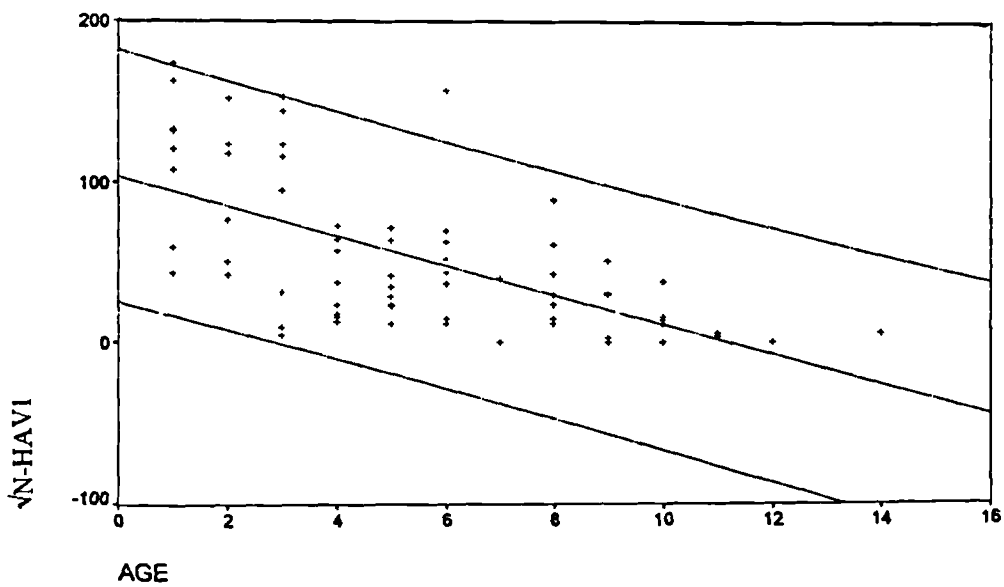


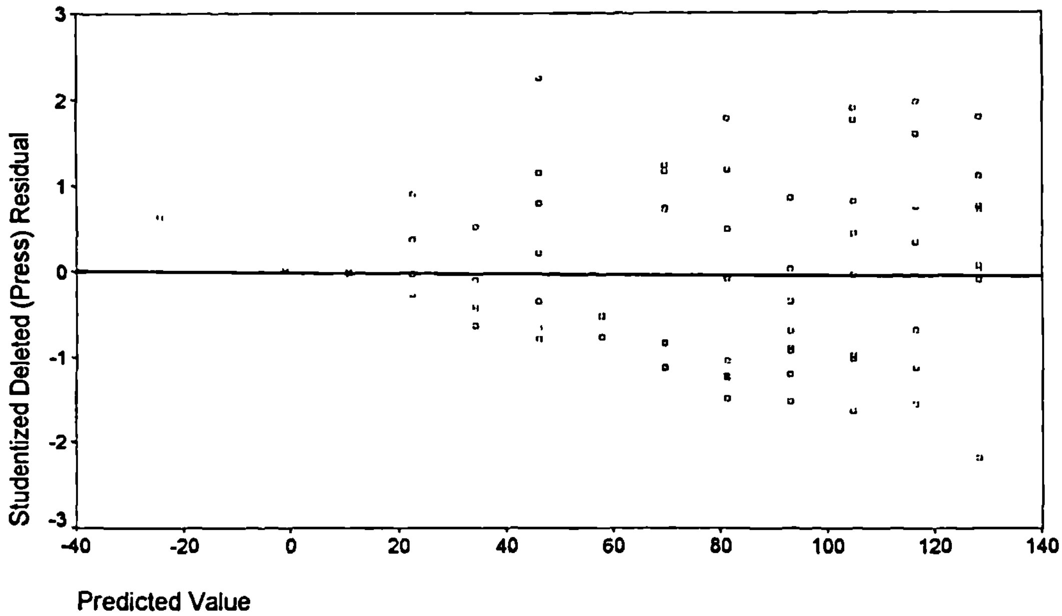
Figure 5.59: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 1 (√N-HAV1) And Cement Layer Age



Regression of non-Haversian canal count in position 2 on cement layer age (n=72):

- The studentised deleted residuals were distributed around 0 (Figure 5.60) but, their distribution was not random, and their variability increased with an increase in predicted count. This indicated that the they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed non-Haversian canal count in position 2 and cement layer age (below).

Figure 5.60: Scatterplot Of Studentised Deleted Resiudals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 2 on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.15) and most points in the Q-Q plot (Figure 5.61) were distributed on, or very close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.62). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.31 (Table 5.15). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.57 (P=0.00) (Table 5.15). This indicated that there was a highly significant weak linear relationship between the two variables.
- The value of r^2 was 0.32 (Table 5.15). This indicated that the model did not fit the data well and, that, only 32% of a change in transformed non-Haversian canal count in position 2 could be accounted for by a change in cement layer age.

Table 5.15: Regression Of Transformed Non-Haversian Canal Count In Position 2 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.74	11.74	-0.57 (P=0.00)	0.32	>0.20	2.31

Figure 5.61: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

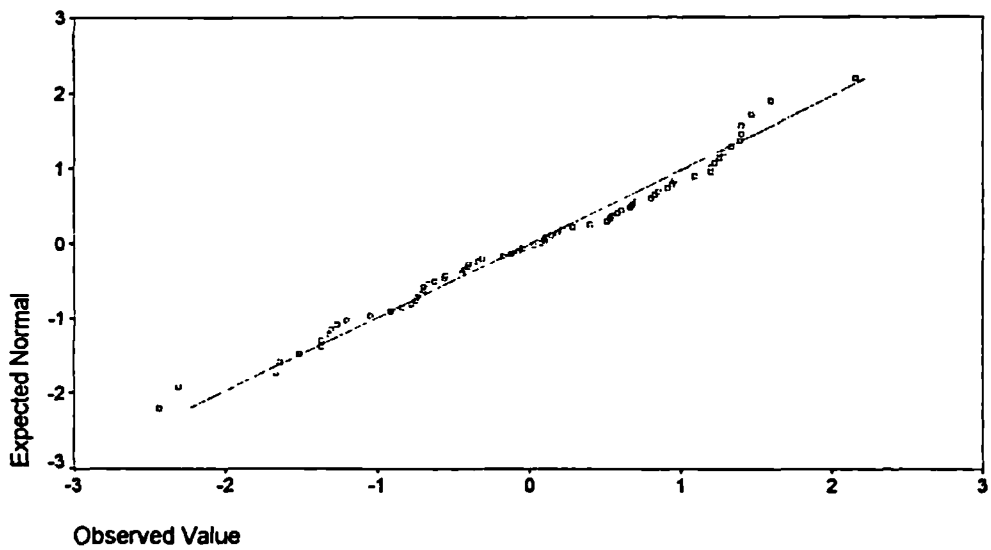


Figure 5.62: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

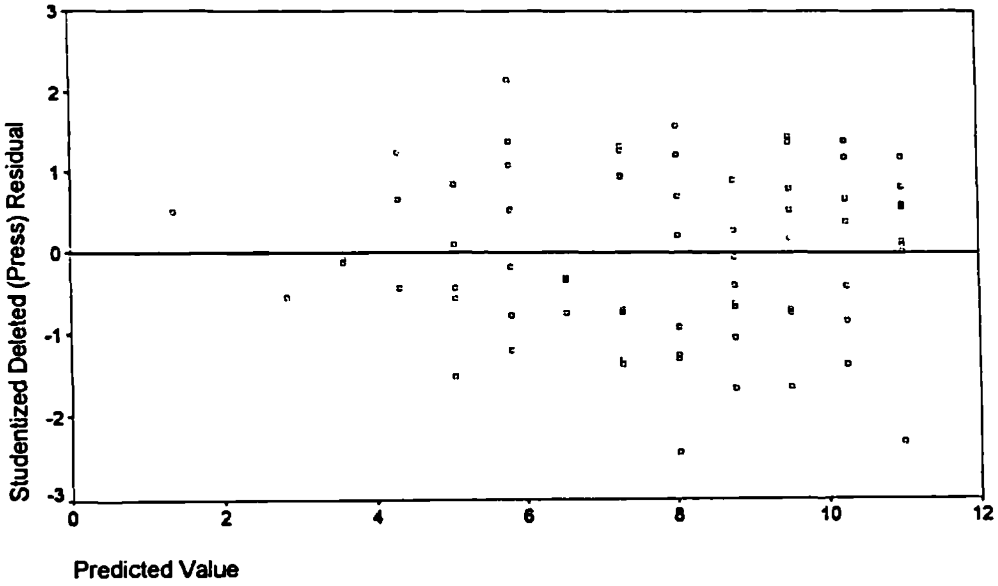
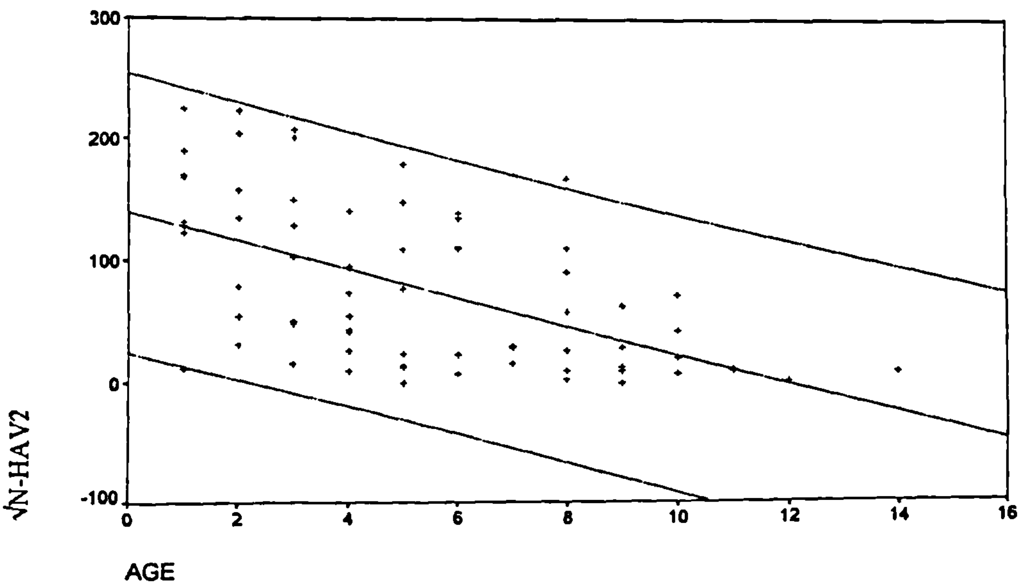


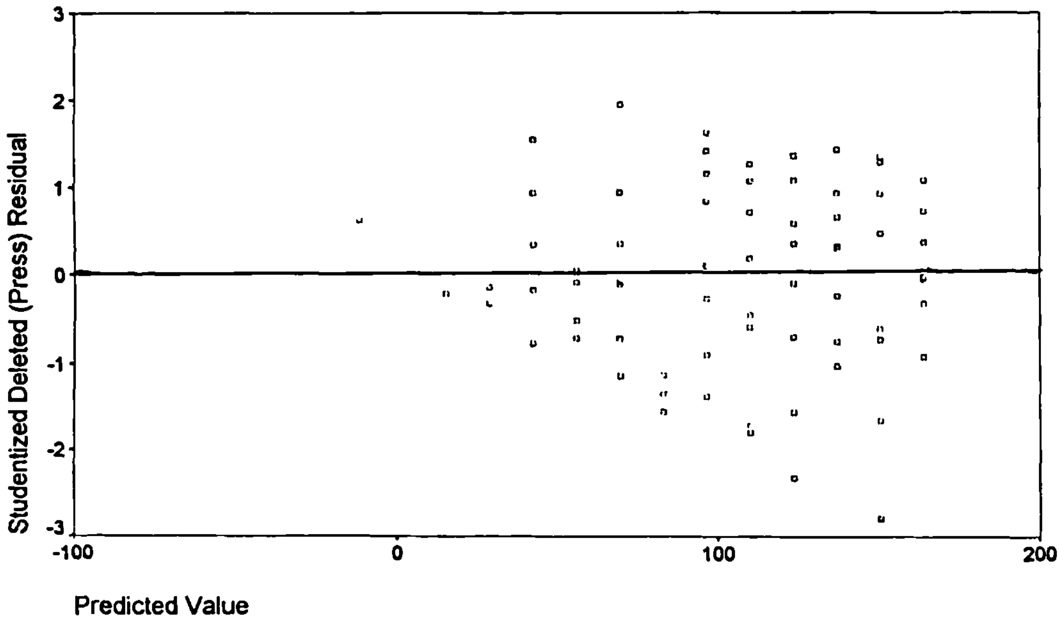
Figure 5.63: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 2 (√N-HAV2) And Cement Layer Age



Regression of non-Haversian canal count in position 3 on cement layer age (n=72):

- The studentised deleted residuals were distributed around 0 (Figure 5.64) but, their distribution was not random, and their variability increased with an increase in predicted count. This indicated that the they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed non-Haversian canal count in position 3 and cement layer age (below).

Figure 5.64: Scatterplot of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 3 on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.16) and most points in the Q-Q plot (Figure 5.65) were distributed on, or very close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.66). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.98 (Table 5.16). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.64 (P=0.00) (Table 5.16). This indicated that there was a highly significant, moderately strong degree of linear association between the two variables.
- The value of r^2 was 0.40 (Table 5.16). This indicated that the model fitted the data moderately well and, that, 40% of a change in transformed non-Haversian canal count in position 3 could be accounted for by a change in cement layer age.

Table 5.16: Regression Of Transformed Non-Haversian Canal Count In Position 3 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.75	13.57	-0.64 (P=0.00)	0.40	>0.20	1.98

Figure 5.65: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

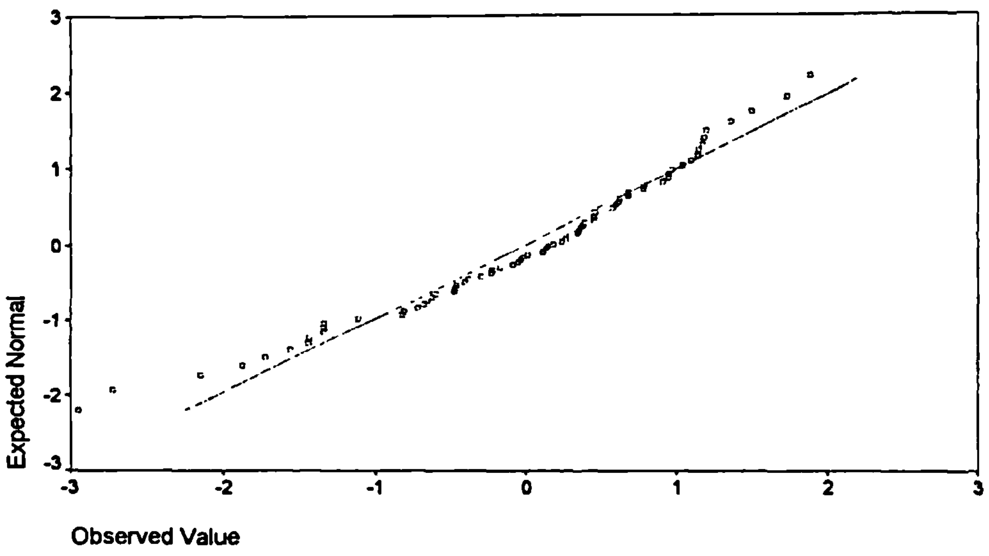


Figure 5.66: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

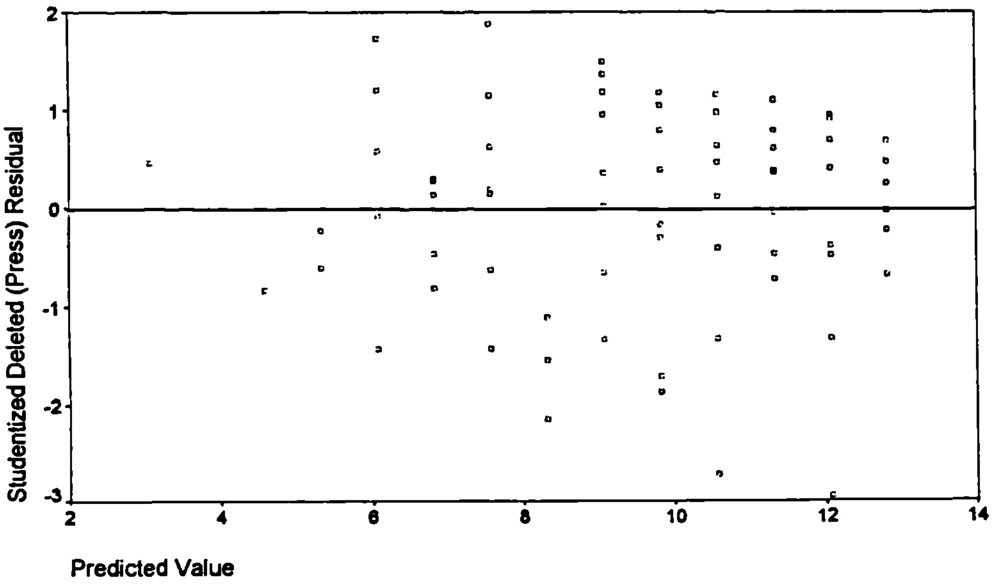
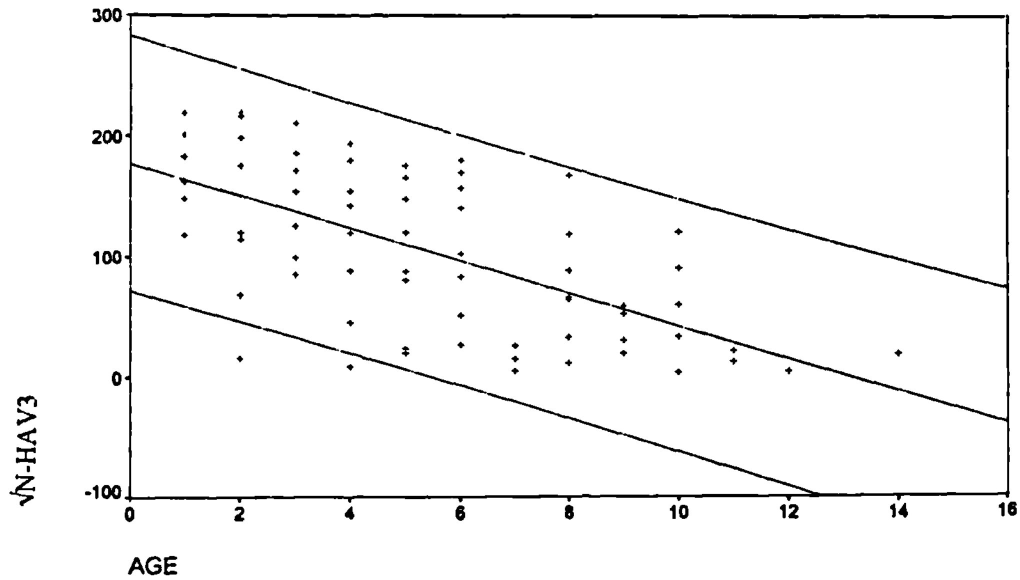


Figure 5.67: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 3 (√N-HAV3) And Cement Layer Age



Regression of non-Haversian canal count in position 4 on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.00. This indicated that the studentised deleted residuals were not normally distributed and that it was not appropriate to fit a linear regression model. Instead the count was transformed and a new regression model was fitted to the relationship between the transformed non-Haversian canal count in position 4 and cement layer age (below).

Regression of transformed non-Haversian canal count in position 4 on cement layer age (n=72):

- The K-S (Lilliefors) significance was 0.04 (Table 5.17) and most points in the Q-Q plot (Figure 5.68) were distributed around a straight line. Although the K-S (Lilliefors) significance was on the borderline of acceptability for normal distribution, the majority of points in the Q-Q plot did fall around a straight line and, as the studentised deleted residuals adhered to all other regression assumptions (below), a linear regression model was fitted.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.69). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.62 (Table 5.17). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.53 (P=0.00) (Table 5.17). This indicated that there was a highly significant weak linear relationship between the two variables.
- The value of r^2 was 0.28 (Table 5.17). This indicated that the model did not fit the data well and, that, only 28% of a change in transformed non-Haversian canal count in position 4 could be accounted for by a change in cement layer age.

Table 5.17: Regression Of Transformed Non-Haversian Canal Count In Position 4 On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.58	9.72	-0.53 (P=0.00)	0.28	0.04	1.62

Figure 5.68: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

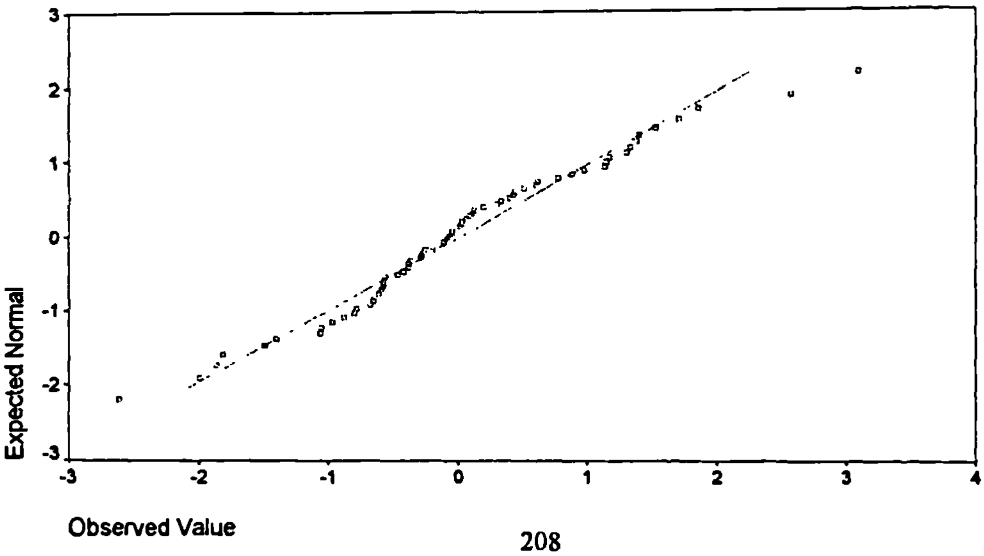


Figure 5.69: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

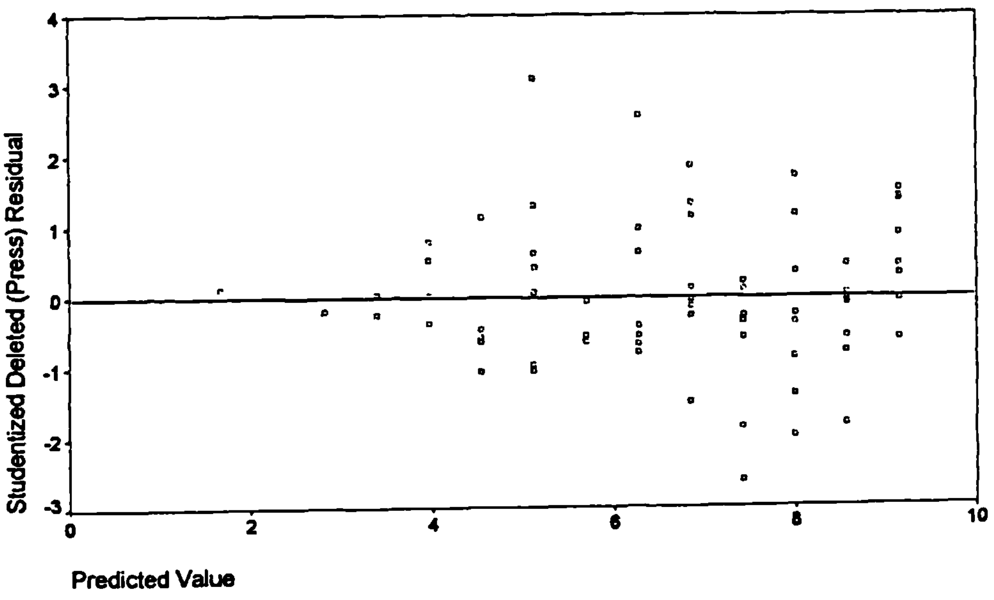
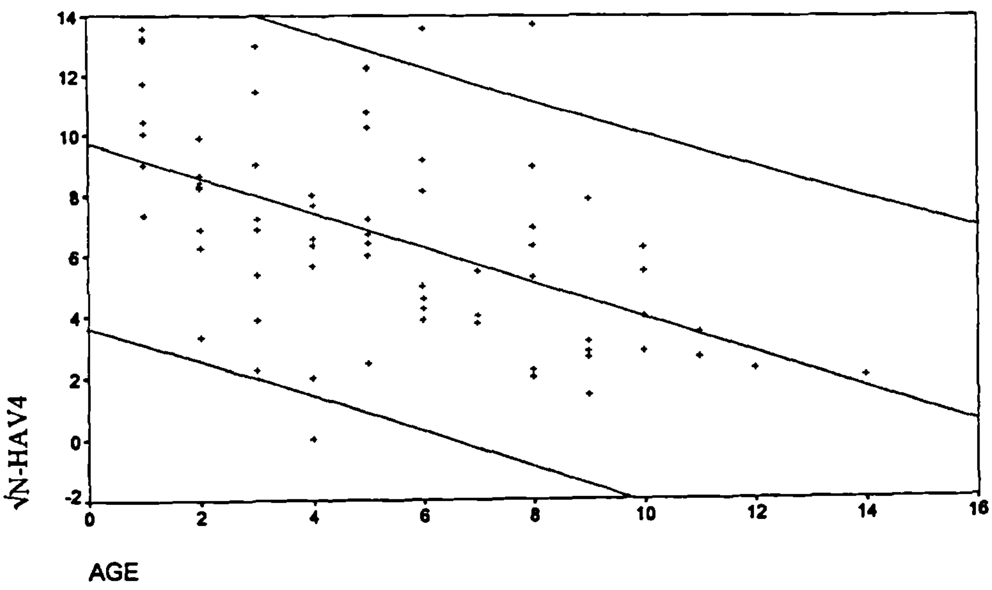


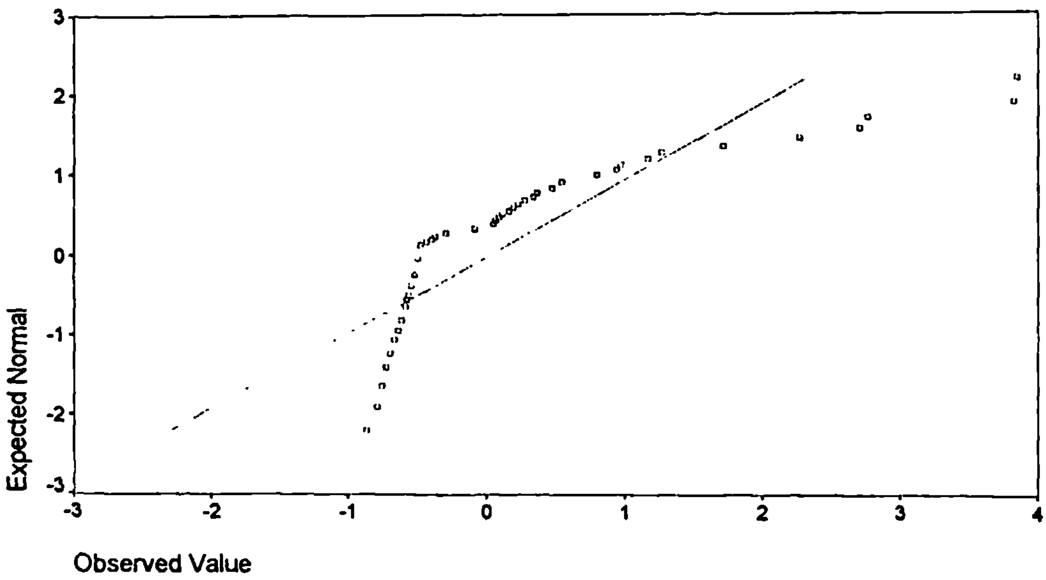
Figure 5.70: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 4 (√N-HAV4) And Cement Layer Age



Regression of total non-Haversian canal count on cement layer age (n=72):

- The majority of points in the Q-Q plot (Figure 5.71) were not distributed around a straight line. This indicated that the studentised deleted residuals were not normally distributed and that it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed total non-Haversian canal count and cement layer age (below).

Figure 5.71: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals



Regression of transformed total non-Haversian canal count on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.18) and most points in the Q-Q plot (Figure 5.72) were distributed on, or very close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.73). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.90 (Table 5.18). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.68 (P=0.00) (Table 5.18). This indicated that there was a highly significant, moderately strong linear relationship between the two variables.
- The value of r^2 was 0.46 (Table 5.18). This indicated that the regression model fitted the data quite well and, that, 46% of a change in transformed total non-Haversian canal count could be accounted for by a change in cement layer age.

Table 5.18: Regression Of Transformed Total Non-Haversian Canal Count On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-1.37	23.18	-0.68 (P=0.00)	0.46	>0.20	1.90

Figure 5.72: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

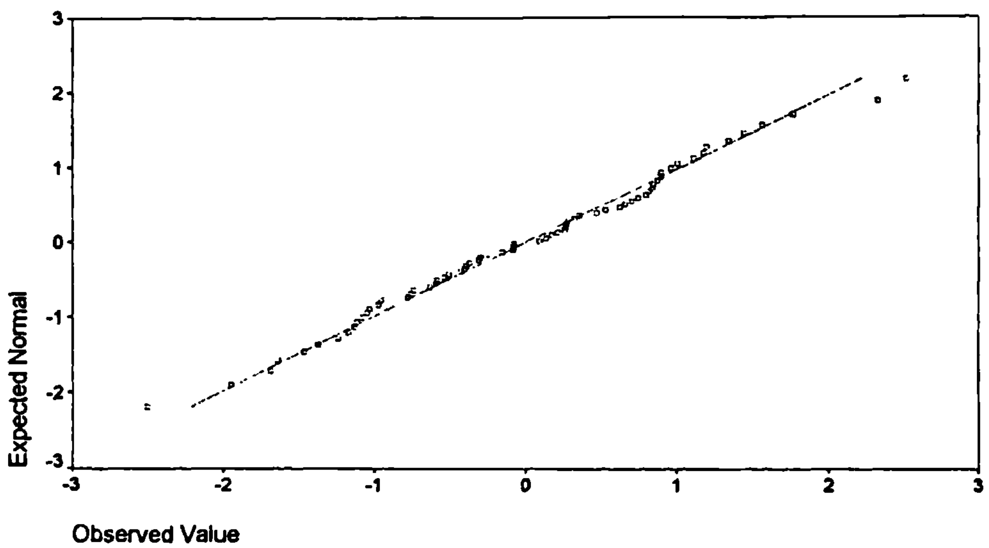


Figure 5.73: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

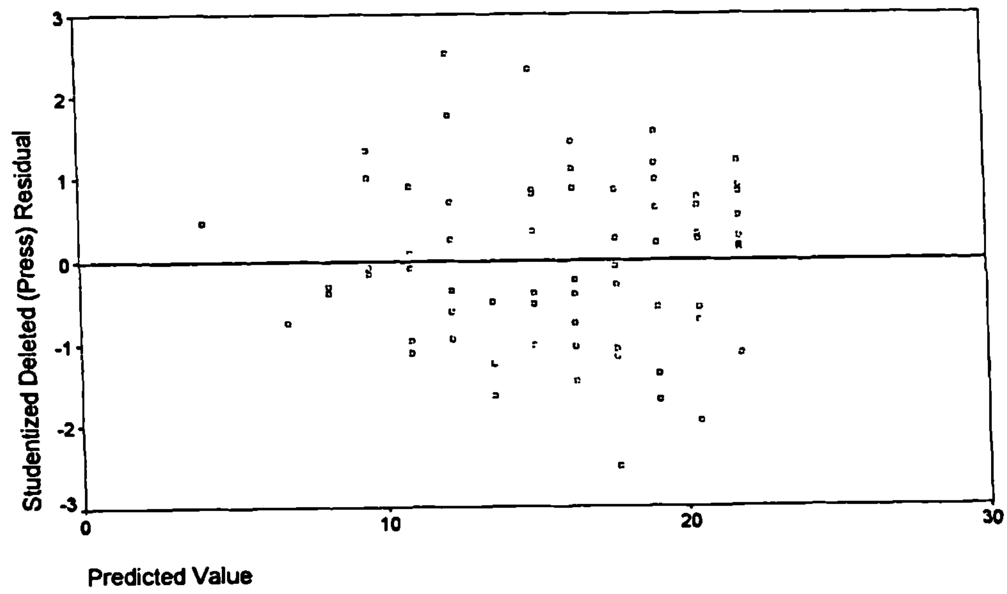
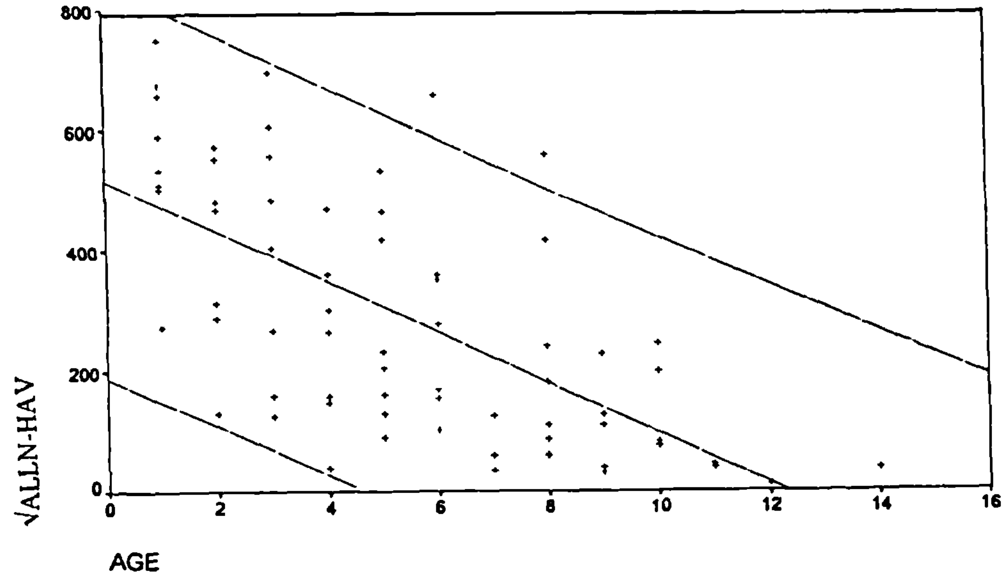


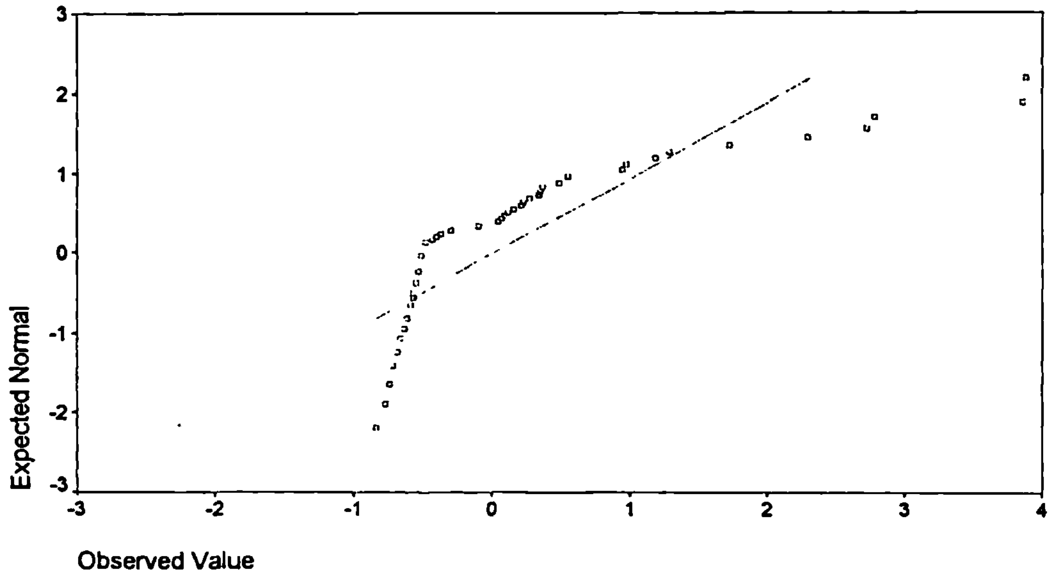
Figure 5.74: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Total Non-Haversian Canal Count (√ALLN-HAV) And Cement Layer Age



Regression of average non-Haversian canal count on cement layer age (n=72):

- The majority of points in the Q-Q plot (Figure 5.75) were not distributed around a straight line. This indicated that the studentised deleted residuals were not normally distributed and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed average non-Haversian canal count and cement layer age (below).

Figure 5.75: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals



Regression of transformed average non-Haversian canal count on cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.19) and most points in the Q-Q plot (Figure 5.76) were distributed on, or very close to, a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.77). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.91 (Table 5.19). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.68 (P=0.00) (Table 5.19). This indicated that there was a highly significant, moderately strong linear relationship between the two variables.
- The value of r^2 was 0.46 (Table 5.19). This indicated that the regression model fitted the data quite well and, that, 46% of a change in transformed average non-Haversian canal count could be accounted for by a change in cement layer age.

Table 5.19: Regression Of Transformed Average Non-Haversian Canal Count On Cement Layer Age (n=72)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.69	11.62	-0.68(P=0.00)	0.46	>0.20	1.91

Figure 5.76: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

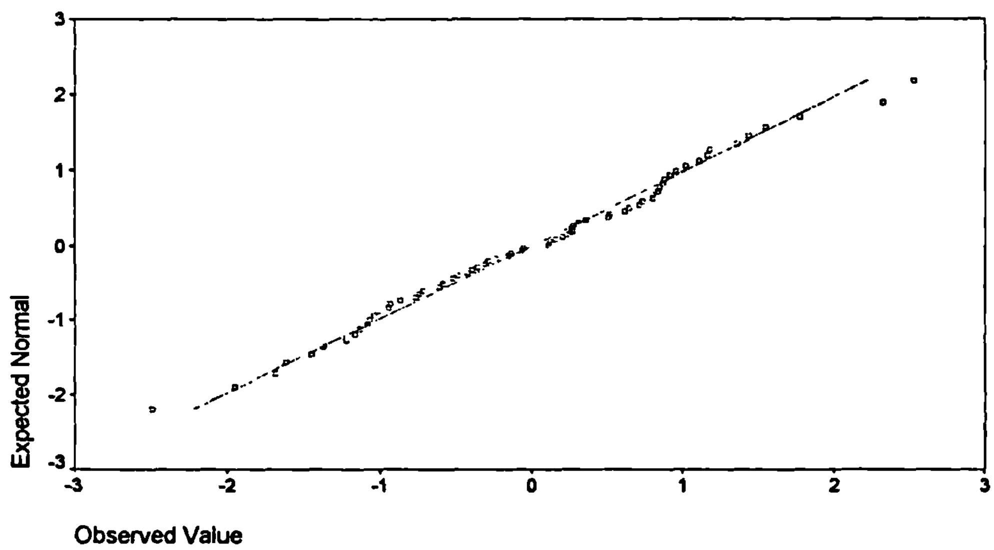


Figure 5.77: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

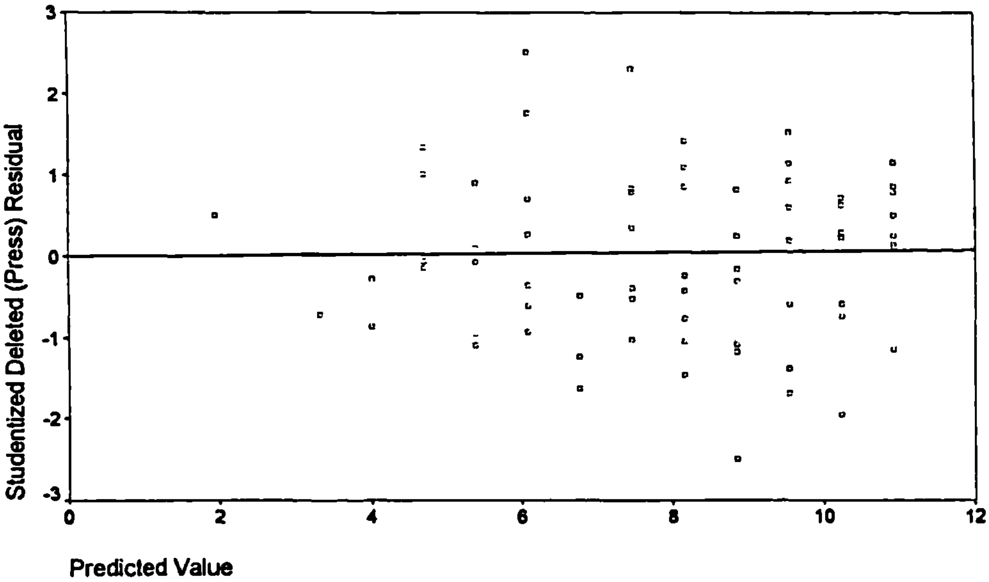
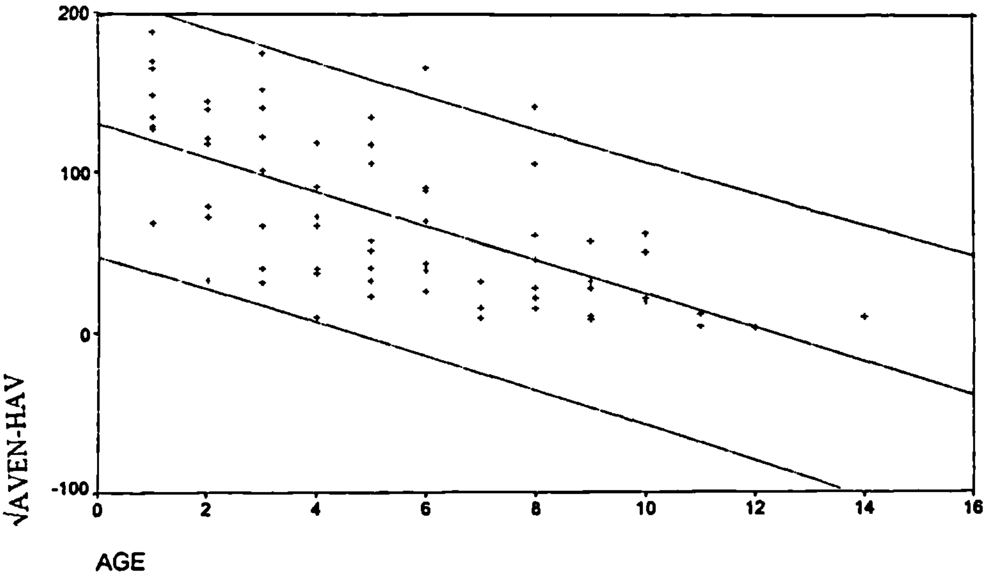


Figure 5.78: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Average Non-Haversian Canal Count (√AVEN-HAV) And Cement Layer Age



5.3.2 THE BUCKS

Regression of secondary osteon count in position 1 on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.20) and the majority of points in the Q-Q plot (Figure 5.79) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.80). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.77 (Table 5.20). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.41 (P=0.03) (Table 5.20). This indicated that there was a significant weak degree of linear association between the two variables.
- The value of r^2 was 0.16 (Table 5.20). This indicated that the regression model did not fit the data well and, that, only 16% of a change in secondary osteon count in position 1 could be accounted for by a change in cement layer age.

Table 5.20: Regression Of Secondary Osteon Count In Position 1 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
2.98	13.32	0.41 (P=0.03)	0.16	>0.20	1.77

Figure 5.79: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

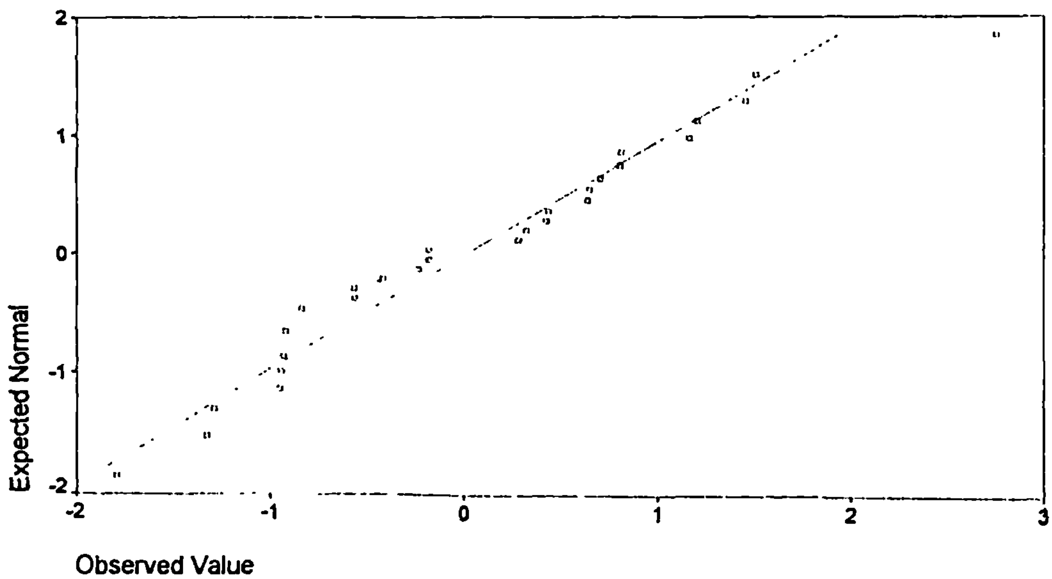


Figure 5.80: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

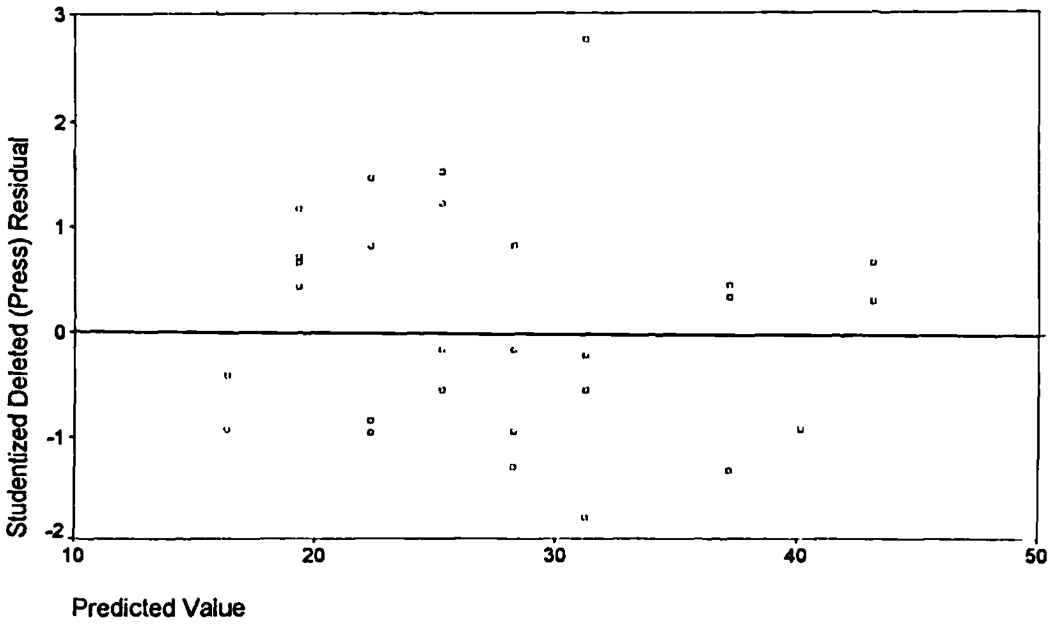
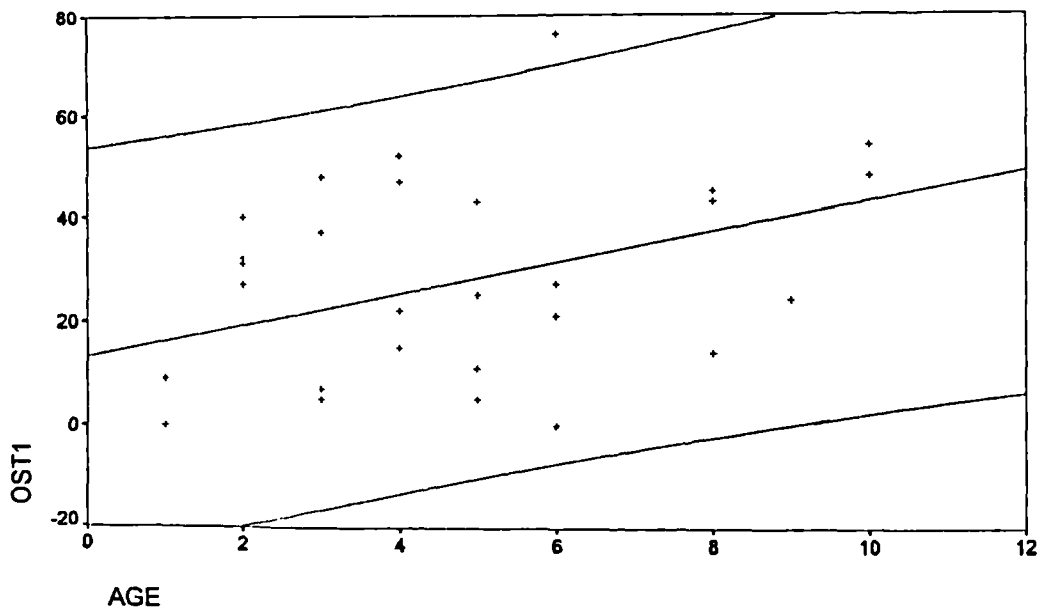


Figure 5.81: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 1 (OST1) And Cement Layer Age



Regression of secondary osteon count in position 2 on cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.10 (Table 5.21) and the majority of points in the Q-Q plot (Figure 5.82) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.83). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.68 (Table 5.21). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.25 (P=0.18) (Table 5.21). This indicated that there was a weak degree of linear association between the two variables.
- The value of r^2 was 0.06 (Table 5.21). This indicated that the regression model did not fit the data well and, that, only 6% of a change in secondary osteon count in position 2 could be accounted for by a change in cement layer age.

Table 5.21: Regression Of Secondary Osteon Count In Position 2 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
2.29	17.17	0.25 (P=0.18)	0.06	0.10	1.68

Figure 5.82: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

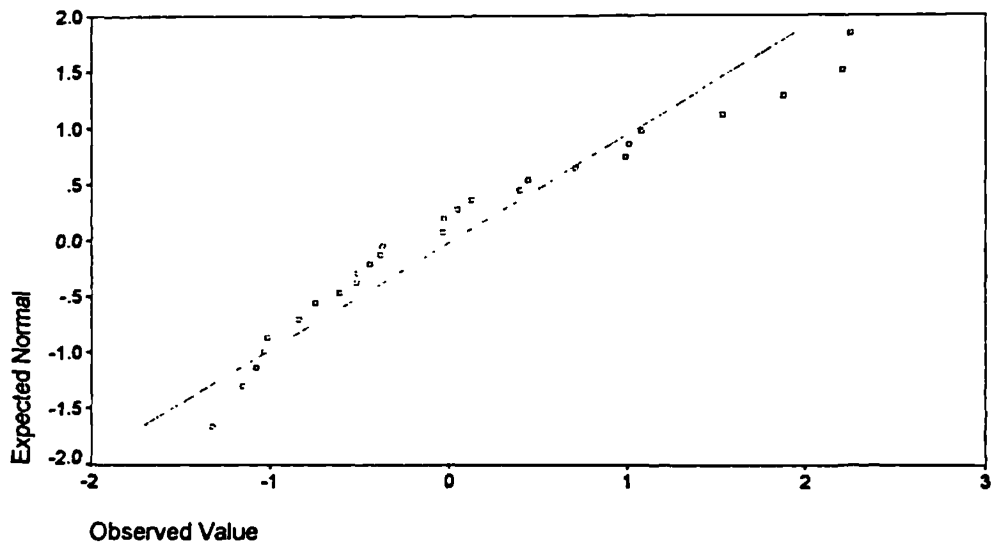


Figure 5.83: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

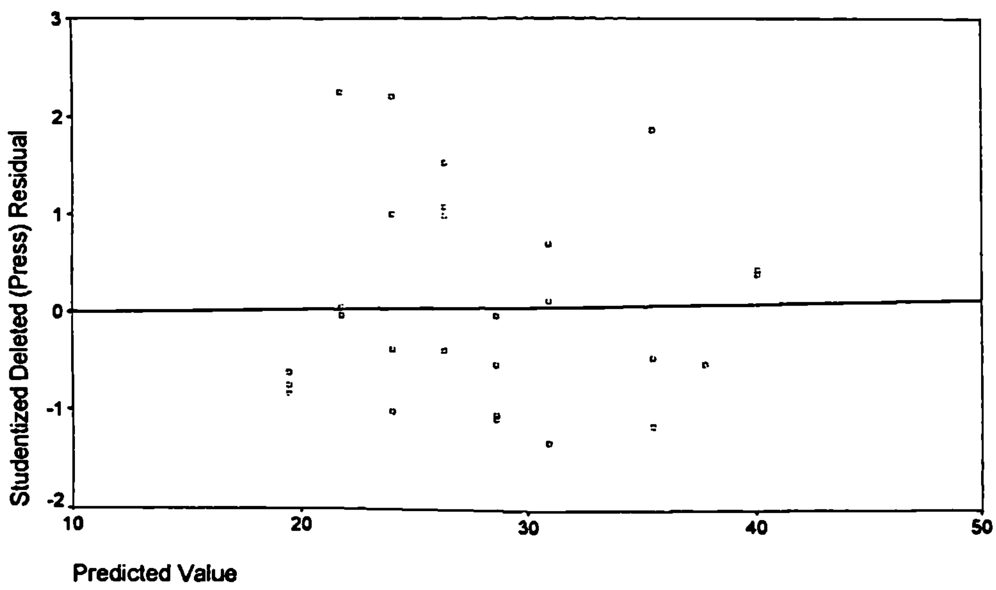
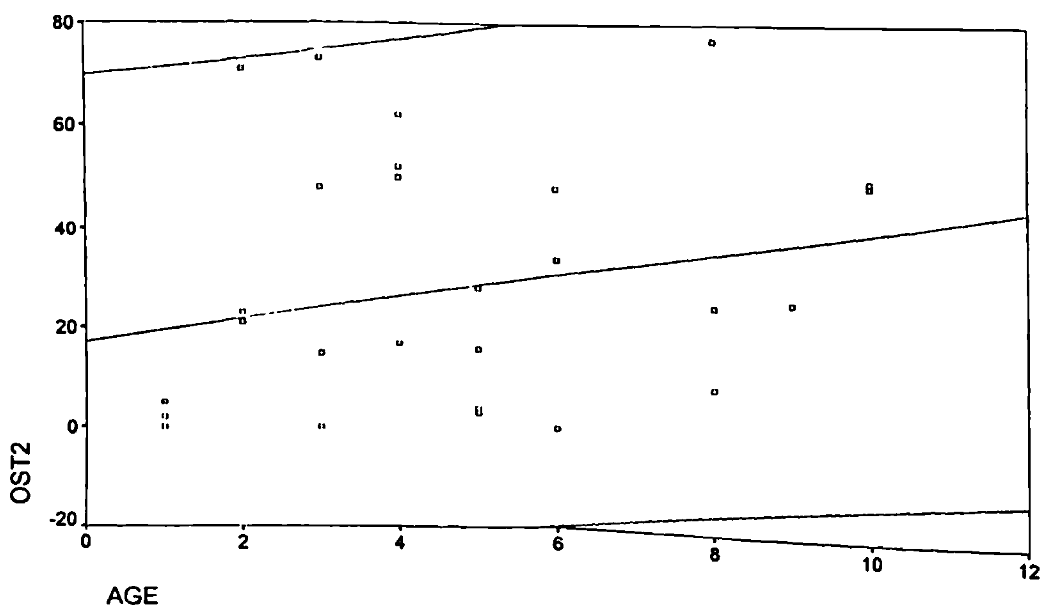


Figure 5.84: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 2 (OST2) And Cement Layer Age



Regression of secondary osteon count in position 3 on cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.00. This indicated that the studentised deleted residuals were not normally distributed and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between the transformed secondary osteon count in position 3 and cement layer age (below).

Regression of transformed secondary osteon count in position 3 and cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.16 (Table 5.22) and the majority of points in the Q-Q plot (Figure 5.85) were distributed around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.86). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.72 (Table 5.22). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.29 (P=0.12) (Table 5.22). This indicated that there was a weak degree of linear association between the two variables.
- The value of r^2 was 0.08 (Table 5.22). This indicated that the regression model did not fit the data well and, that, only 8% of a change in transformed secondary osteon count in position 3 could be accounted for by a change in cement layer age.

Table 5.22: Regression Of Transformed Secondary Osteon Count In Position 3 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
0.30	1.70	0.29 (P=0.12)	0.08	0.16	1.72

Figure 5.85: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

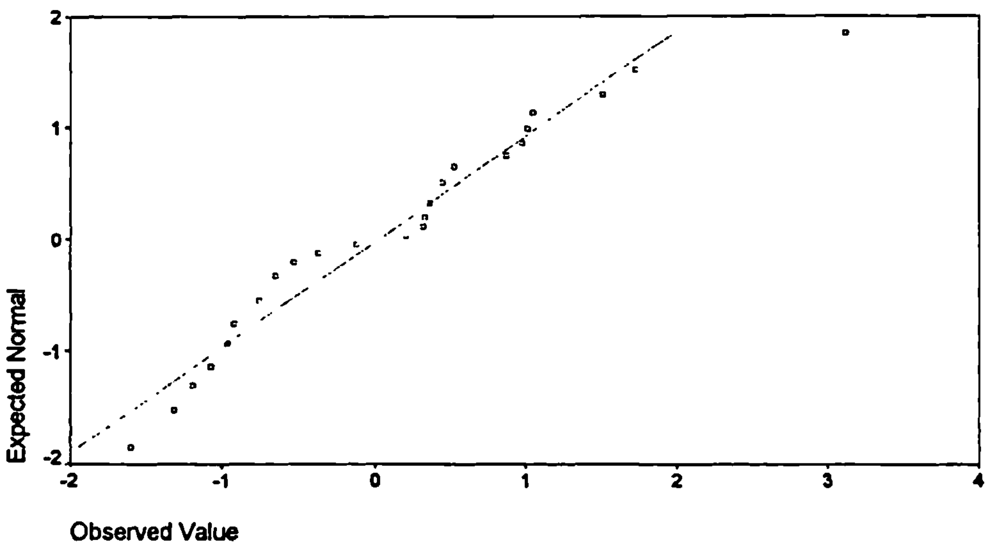


Figure 5.86: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

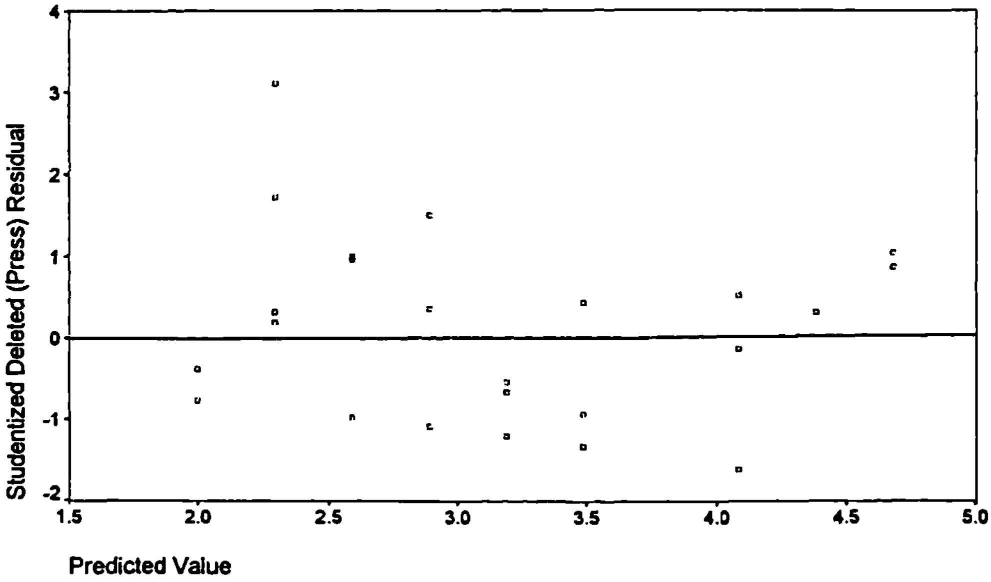
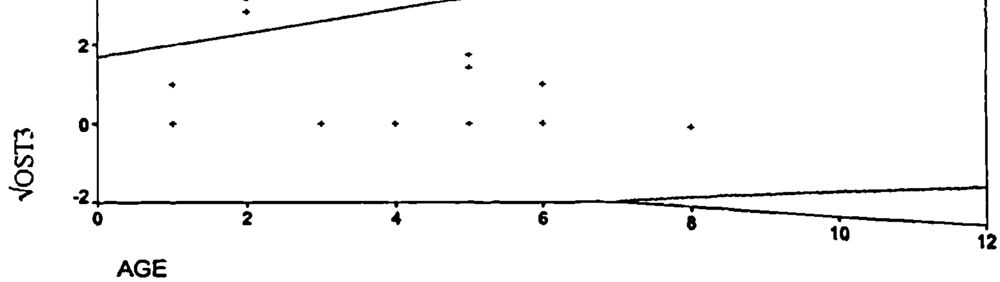


Figure 5.87: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Secondary Osteon Count In Position 3 (√OST3) And Cement Layer Age



Regression of secondary osteon count in position 4 on cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.16 (Table 5.23) and the majority of points in the Q-Q plot (Figure 5.88) were distributed around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.89). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.10 (Table 5.23). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.15 (P=0.44) (Table 5.23). This indicated that there was no association between the two variables.
- The value of r^2 was 0.02 (Table 5.23). This indicated that the regression model did not fit the data well and, that, only 2% of a change in secondary osteon count in position 4 could be accounted for by a change in cement layer age.

Table 5.23: Regression Of Secondary Osteon Count In Position 4 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
1.22	21.35	0.15 (P=0.44)	0.02	0.16	2.10

Figure 5.88: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

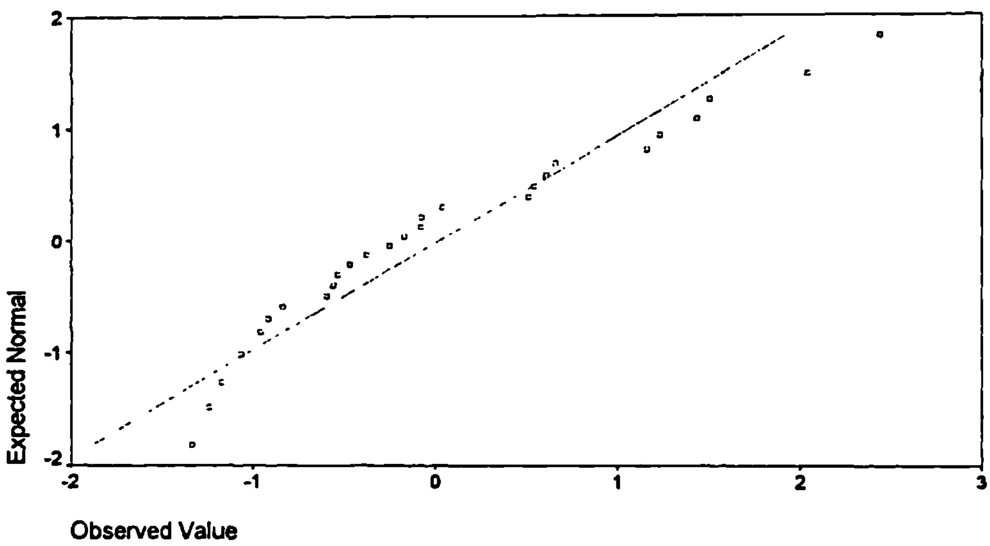


Figure 5.89: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

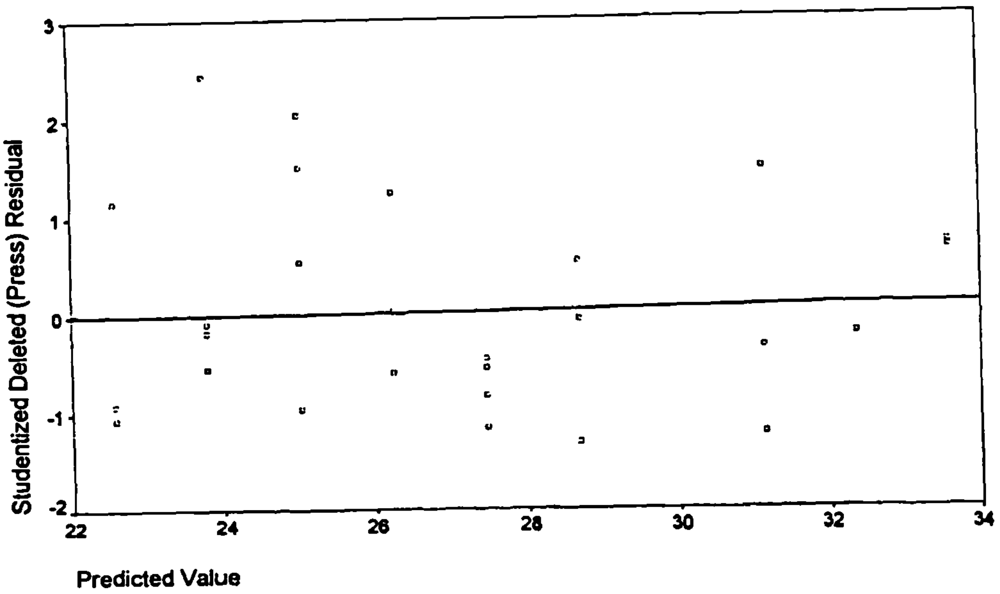
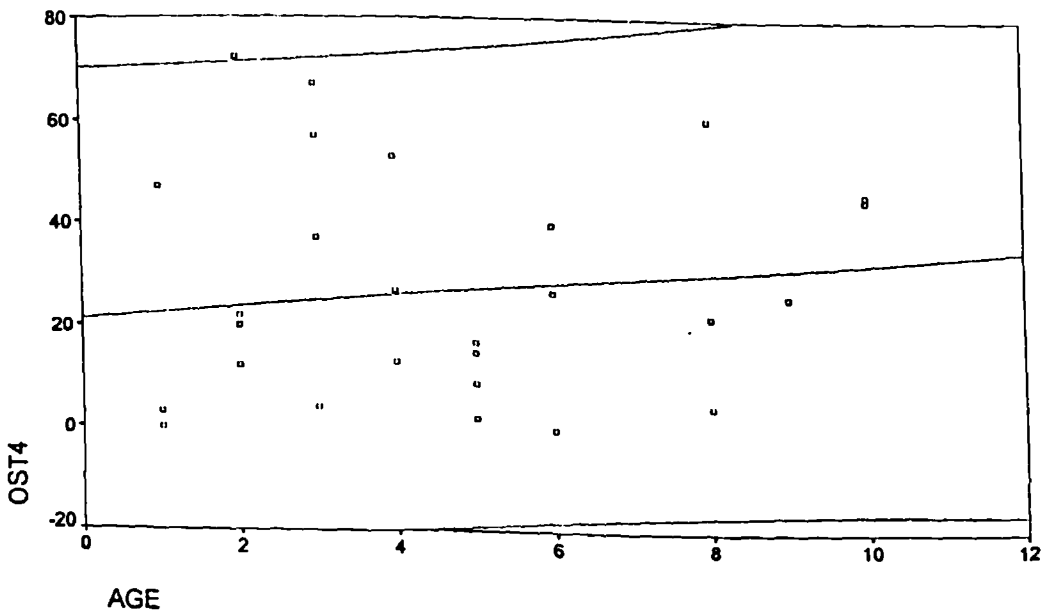


Figure 5.90: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 4 (OST4) And Cement Layer Age



Regression of total secondary osteon count on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.24) and the majority of points in the Q-Q plot (Figure 5.91) were distributed around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.92). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.70 (Table 5.24). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.31 (P=0.11) (Table 5.24). This indicated that there was no association between the two variables.
- The value of r^2 was 0.09 (Table 5.24). This indicated that the regression model did not fit the data well and, that, only 9% of a change in total secondary osteon count could be accounted for by a change in cement layer age.

Table 5.24: Regression Of Total Secondary Osteon Count On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
8.35	58.61	0.31 (P=0.11)	0.09	>0.20	1.70

Figure 5.91: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

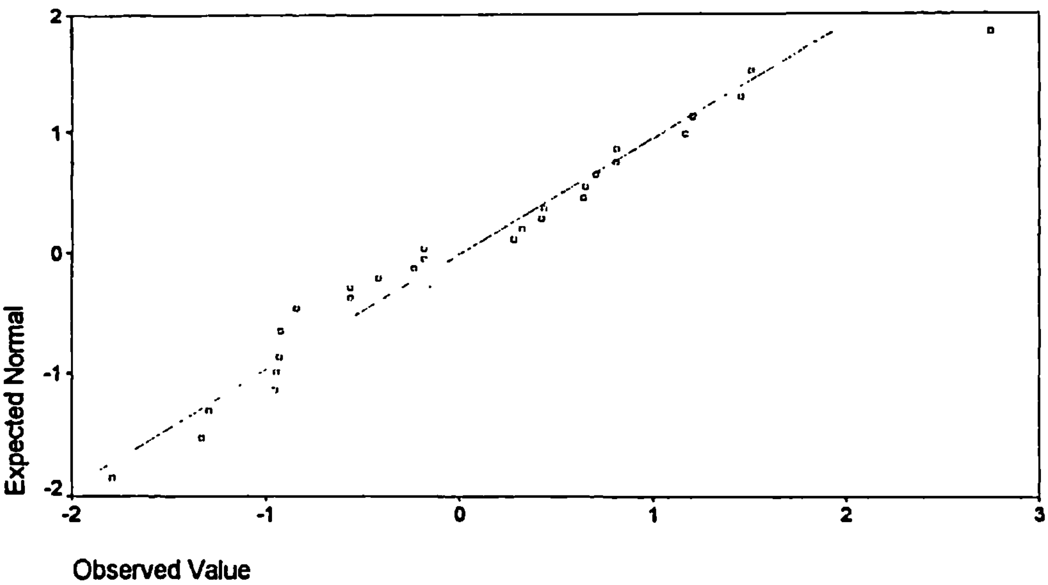


Figure 5.92: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

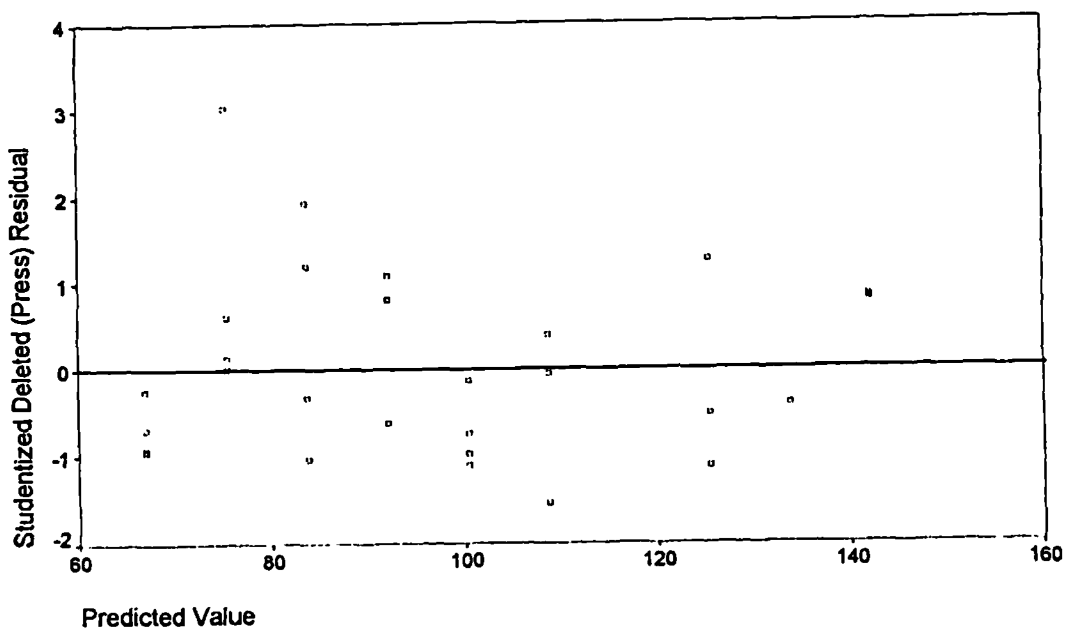
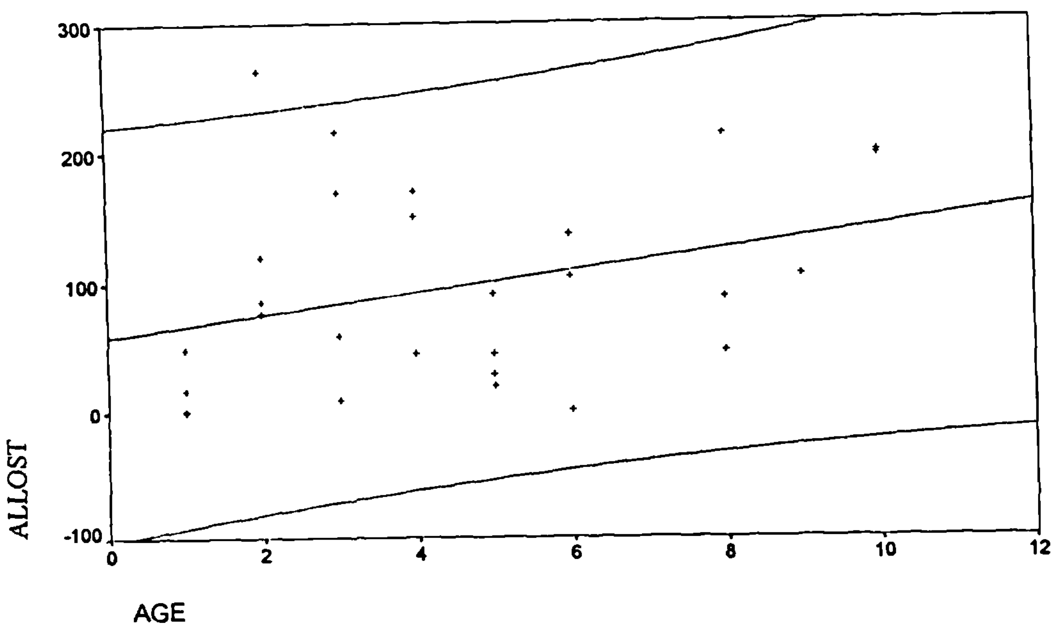


Figure 5.93: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Total Secondary Osteon Count (ALLOST) And Cement Layer Age



Regression of average secondary osteon count on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.25) and the majority of points in the Q-Q plot (Figure 5.94) were distributed around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.95). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.82 (Table 5.25). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.32 (P=0.10) (Table 5.25). This indicated that there was no association between the two variables.
- The value of r^2 was 0.10 (Table 5.25). This indicated that the regression model did not fit the data well and, that, only 10% of a change in average secondary osteon count could be accounted for by a change in cement layer age.

Table 5.25: Regression Of Average Secondary Osteon Count On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
2.26	14.75	0.32 (P=0.10)	0.10	>0.20	1.82

Figure 5.94: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

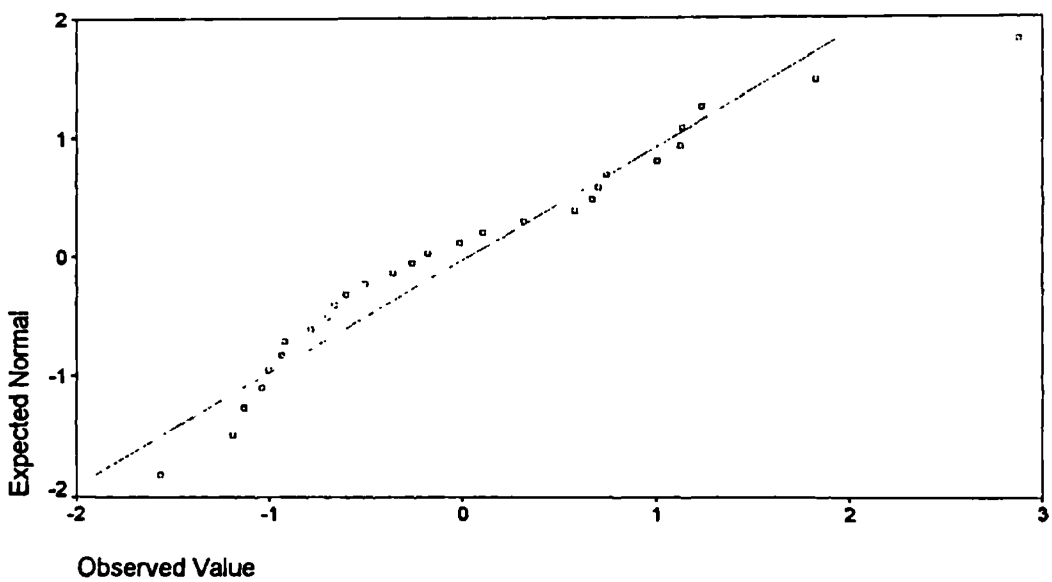


Figure 5.95: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

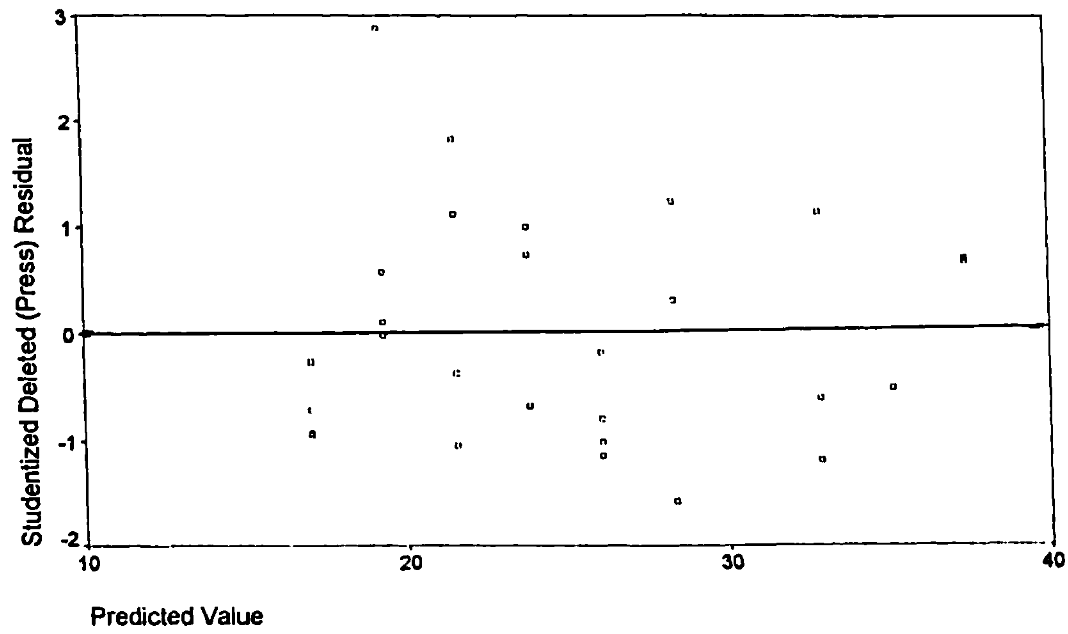
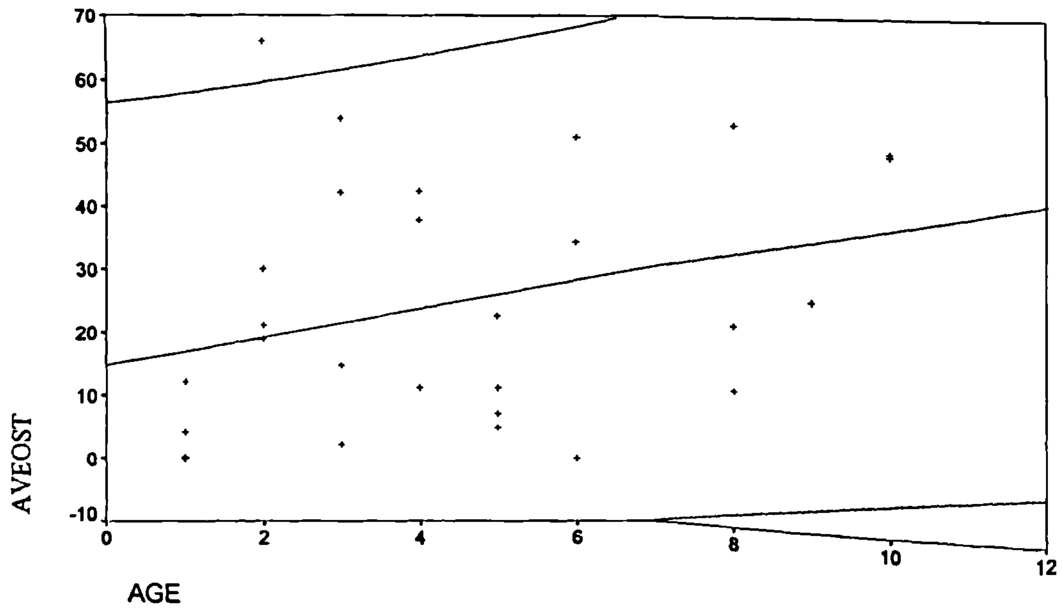


Figure 5.96: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Average Secondary Osteon Count (AVEOST) And Cement Layer Age



Regression of non-Haversian canal count in position 1 on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.26) and the majority of points in the Q-Q plot (Figure 5.97) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.98). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.82 (Table 5.26). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.40 (P=0.03) (Table 5.26). This indicated that there was a significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.16 (Table 5.26). This indicated that the model did not fit the data well and, that, only 16% of a change in non-Haversian canal in position 1 could be accounted for by a change in cement layer age.

Table 5.26: Regression Of Non-Haversian Count In Position 1 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-6.43	90.99	-0.40 (P=0.03)	0.16	>0.20	1.82

Figure 5.97: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

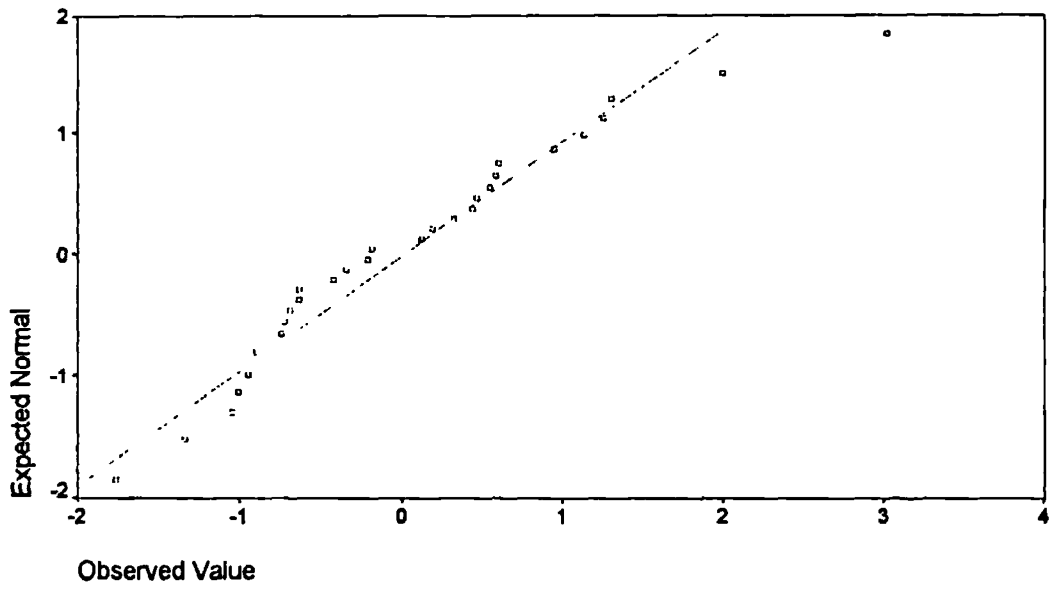


Figure 5.98: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

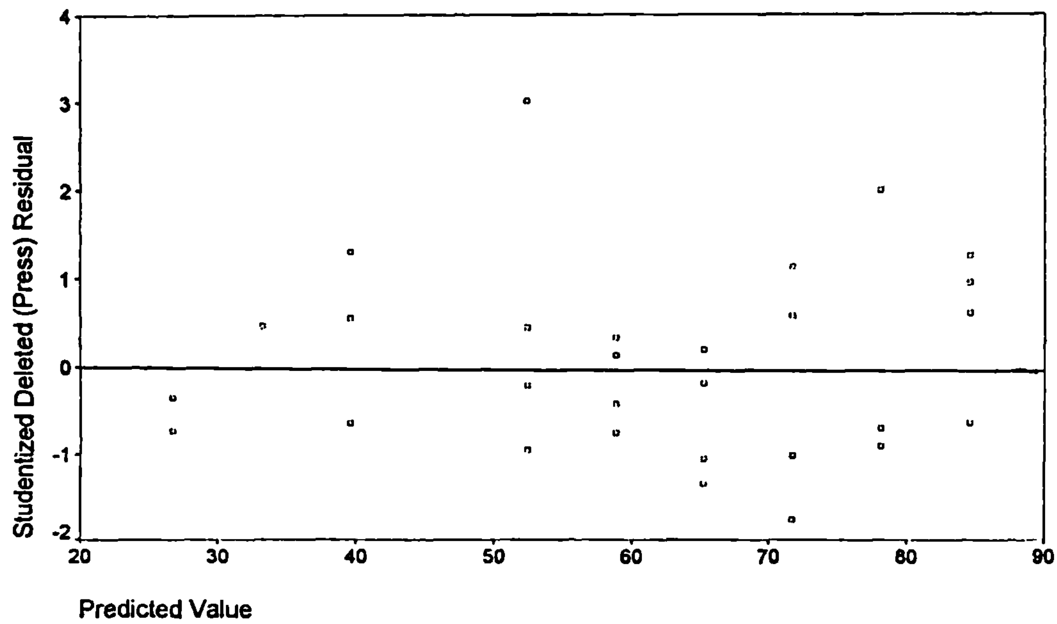
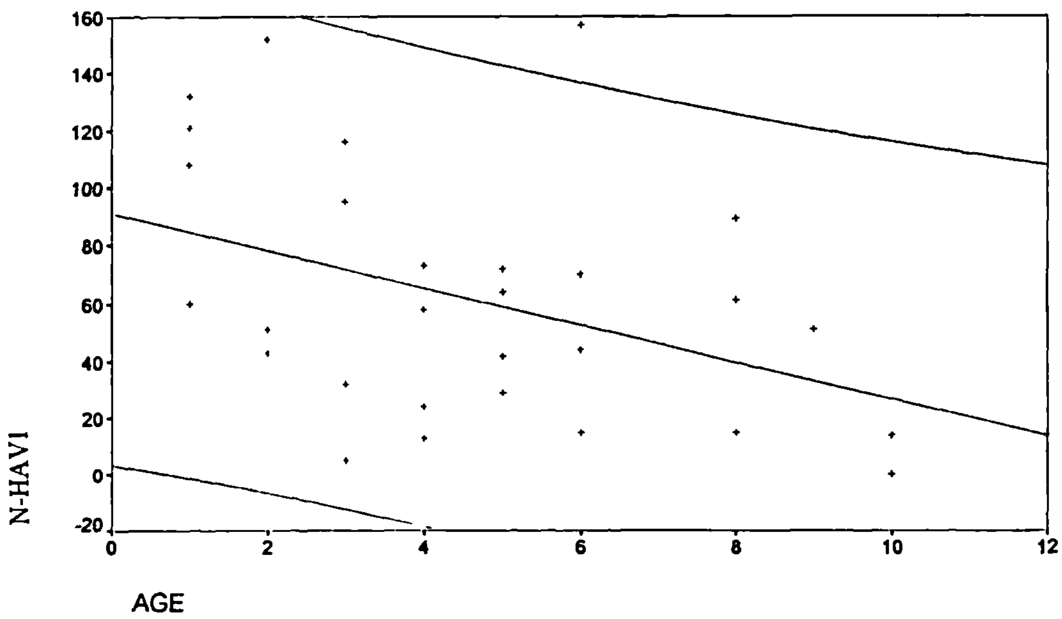


Figure 5.99: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Non-Haversian Canal Count In Position 1 (N-HAV1) And Cement Layer Age



Regression of non-Haversian canal count in position 2 on cement layer age for the bucks (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.27) and the majority of points in the Q-Q plot (Figure 5.100) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.101). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.43 (Table 5.27). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.42 (P=0.02) (Table 5.27). This indicated that there was a significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.18 (Table 5.27). This indicated that the model did not fit the data well and, that, only 18% of a change in non-Haversian canal count in position 2 could be accounted for by a change in cement layer age.

Table 5.27: Regression Of Non-Haversian Count In Position 2 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-9.85	141.24	-0.42 (P=0.02)	0.18	>0.20	2.43

Figure 5.100: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

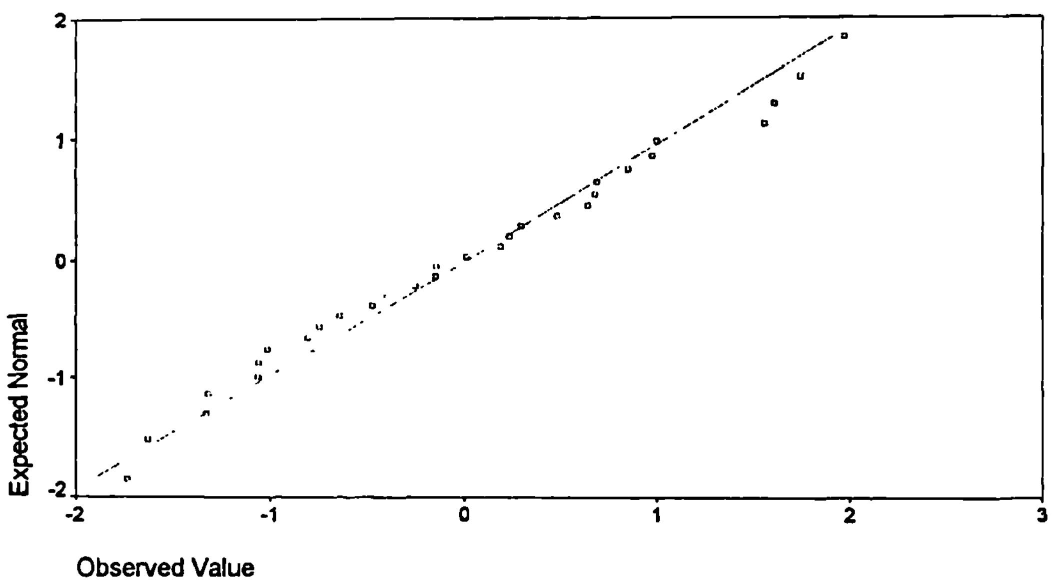


Figure 5.101: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

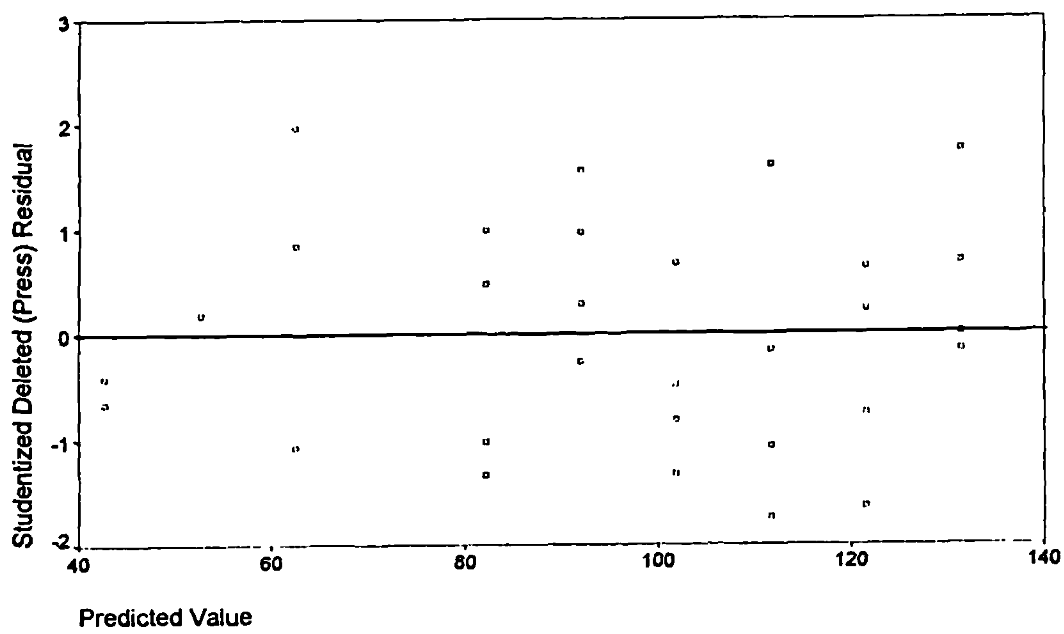
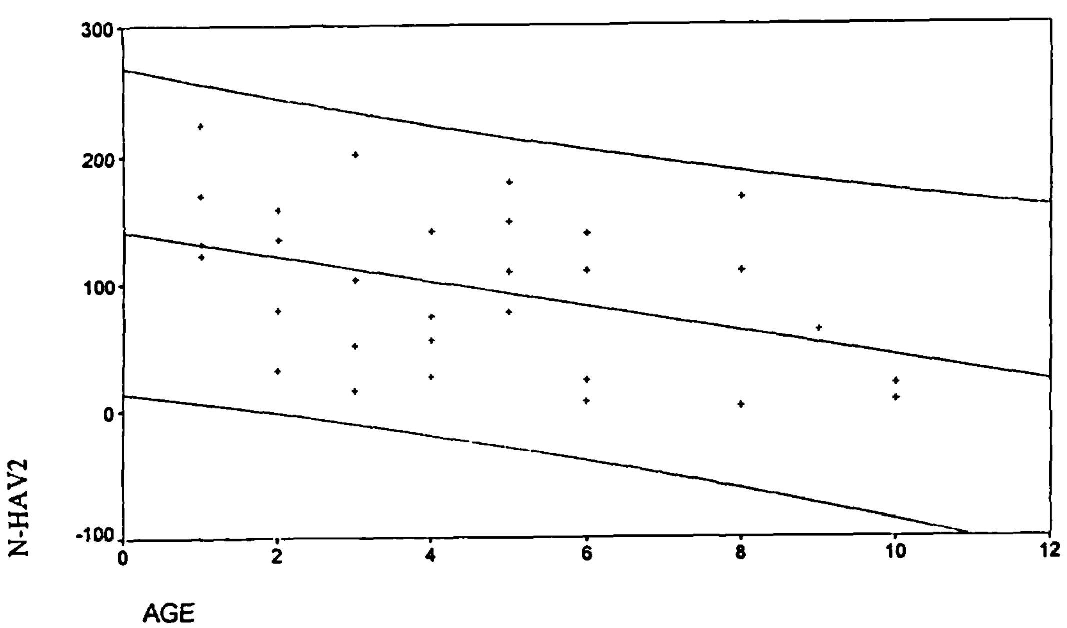


Figure 5.102: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Non-Haversian Canal Count In Position 2 (N-HAV2) And Cement Layer Age



Regression of non-Haversian canal count in position 3 on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.28) and the majority of points in the Q-Q plot (Figure 5.103) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.104). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.20 (Table 5.28). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.44 (P=0.02) (Table 5.28). This indicated that there was a significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.19 (Table 5.28). This indicated that the model did not fit the data well and, that, 19% of a change in non-Haversian canal count in position 3 could be accounted for by a change in cement layer age.

Table 5.28: Regression Of Non-Haversian Count In Position 3 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-9.18	173.19	-0.44 (P=0.02)	0.19	>0.20	2.20

Figure 5.103: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

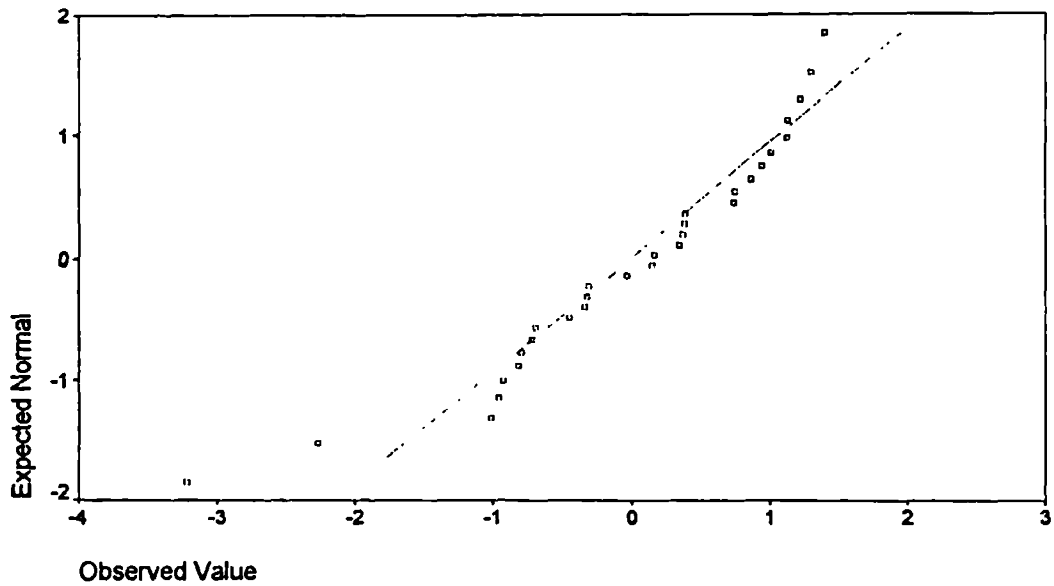


Figure 5.104: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

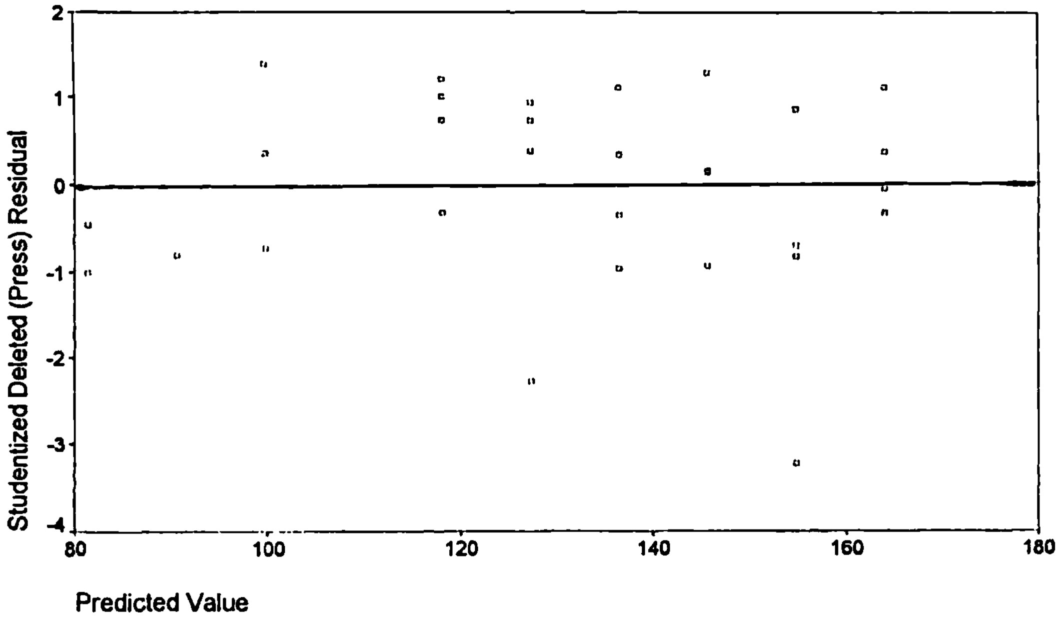
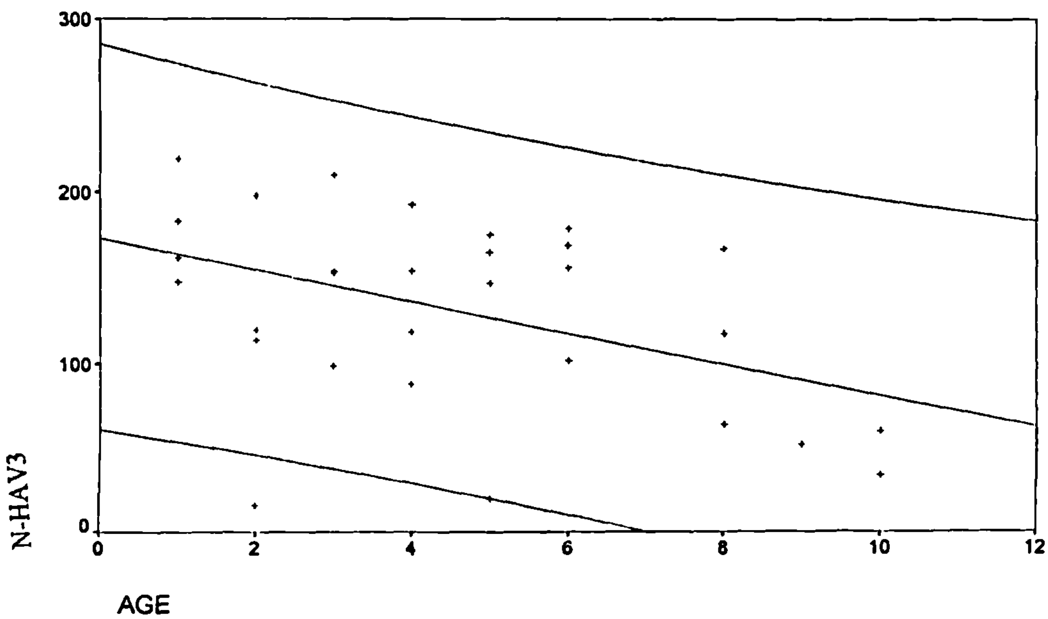


Figure 5.105: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Non-Haversian Canal Count In Position 3 (N-HAV3) And Cement Layer Age



Regression of non-Haversian canal count in position 4 on cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.08 (Table 5.29) and the majority of points in the Q-Q plot (Figure 5.106) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.107). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.55 (Table 5.29). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.21 (P=0.28) (Table 5.29). This indicated that there was a insignificant, weak degree of association between the two variables.
- The value of r^2 was 0.05 (Table 5.29). This indicated that the regression model did not fit the data well and, that, only 5% of a change in non-Haversian canal count in position 4 can be accounted for by a change in cement layer age.

Table 5.29: Regression Of Non-Haversian Count In Position 4 On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-4.42	96.99	-0.21 (P=0.28)	0.05	0.08	1.55

Figure 5.106: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

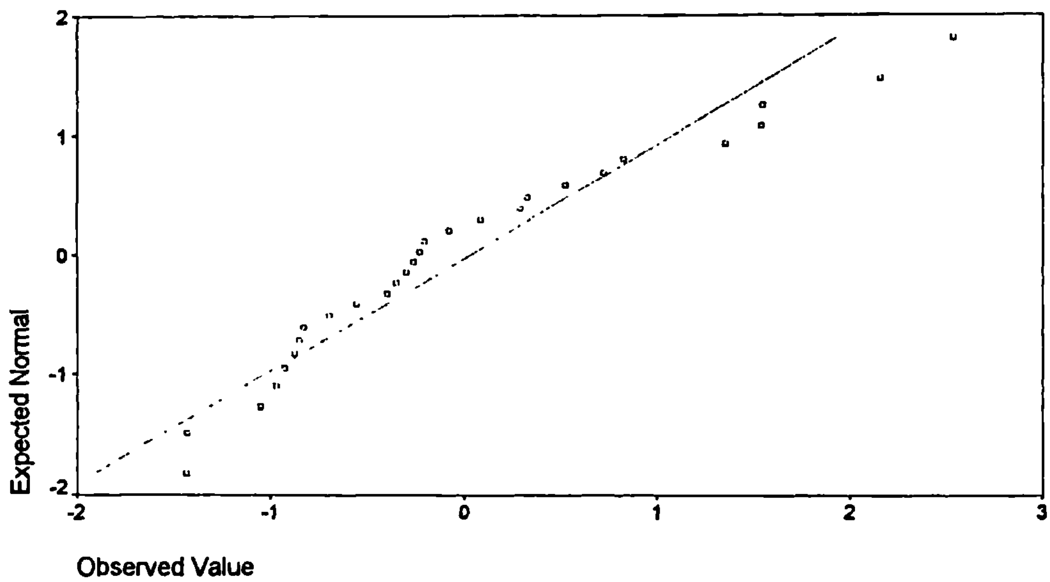


Figure 5.107: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

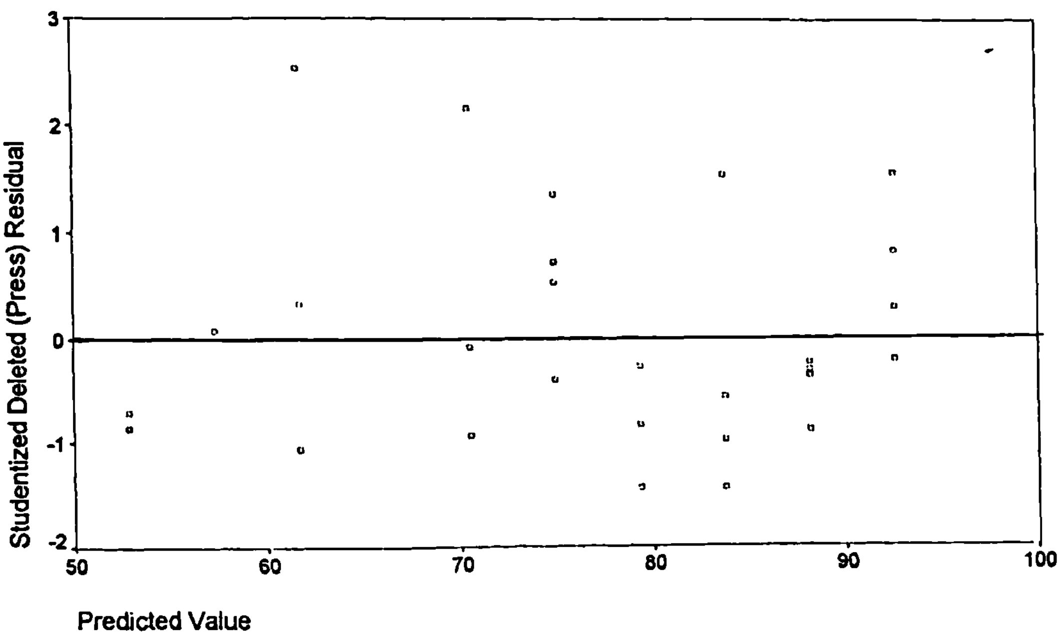
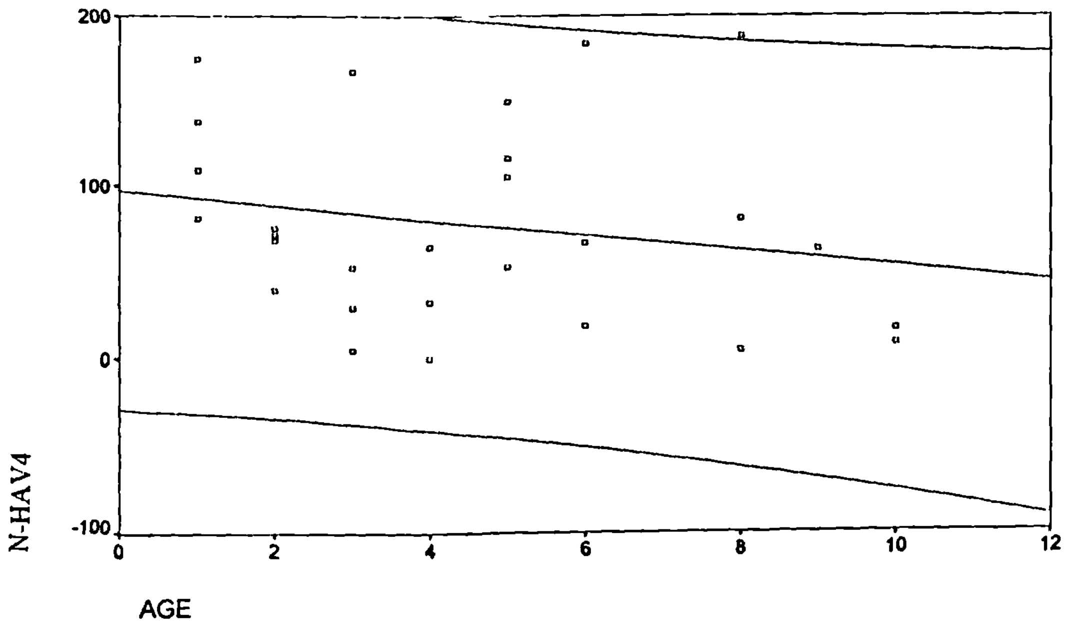


Figure 5.108: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Non-Haversian Canal Count In Position 4 (N-HAV4) And Cement Layer Age



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Regression of total non-Haversian canal count on cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.17 (Table 5.30) and the majority of points in the Q-Q plot (Figure 5.109) did fall around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.110). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.78 (Table 5.30). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.44 (P=0.02) (Table 5.30). This indicated that there was a significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.19 (Table 5.30). This indicated that the regression model did not fit the data well and, that, only 19% of a change in total secondary osteon count could be accounted for by a change in cement layer age.

Table 5.30: Regression Of Total Non-Haversian Count On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-30.07	506.37	-0.44 (P=0.02)	0.19	0.17	1.78

Figure 5.109: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

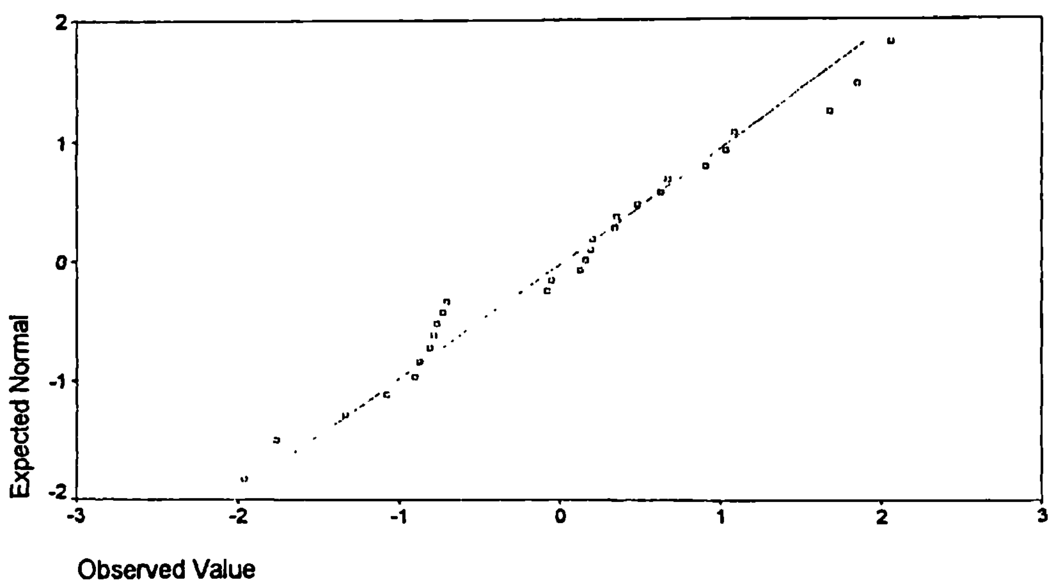


Figure 5.110: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

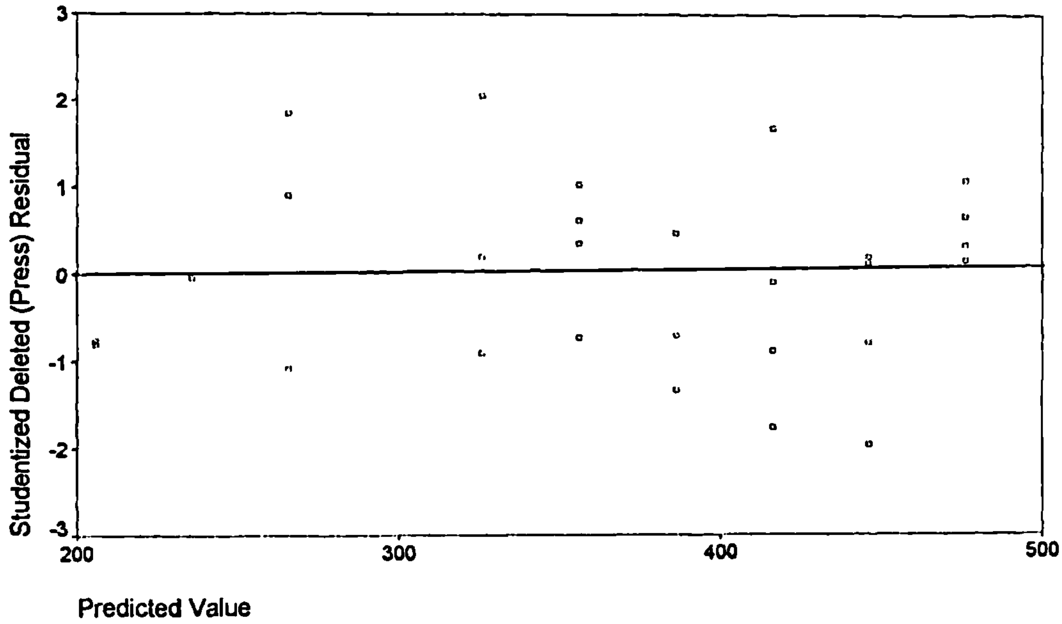
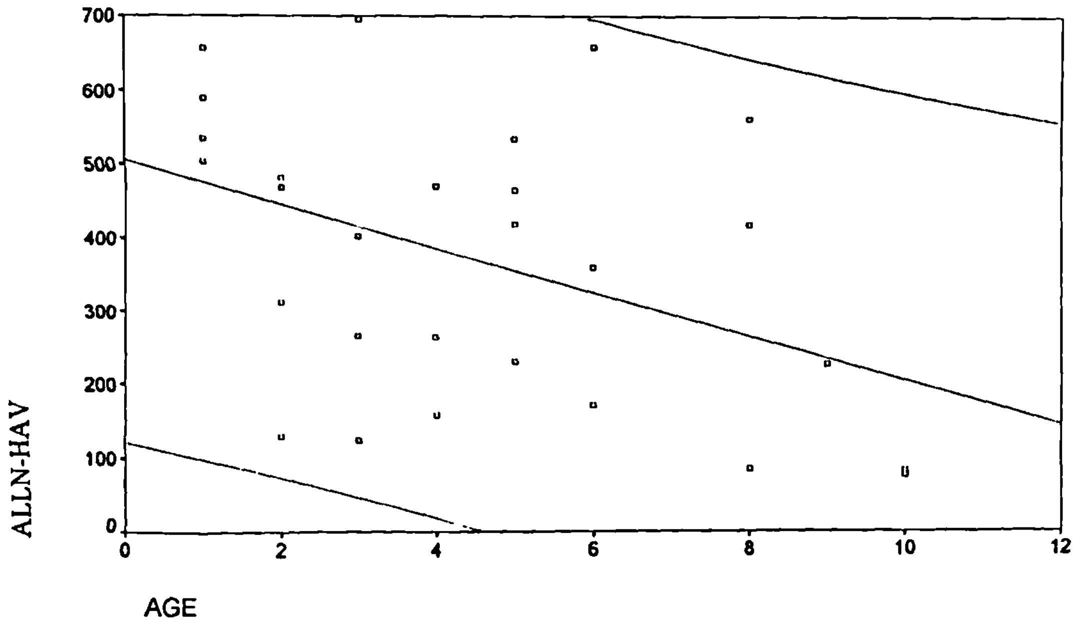


Figure 5.111: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Total Non-Haversian Canal Count (ALLOST) And Cement Layer Age



Regression of average non-Haversian canal count on cement layer age (n=30):

- The K-S (Lilliefors) significance was 0.17 (Table 5.31) and the majority of points in the Q-Q plot (Figure 5.112) fell around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.113). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.78 (Table 5.31). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.44 (P=0.02) (Table 5.31). This indicated that there was a significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.19 (Table 5.31). This indicated that the regression model did not fit the data well and, that, only 19% of a change in average secondary osteon count could be accounted for by a change in cement layer age.

Table 5.31: Regression Of Average Non-Haversian Count On Cement Layer Age (n=30)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-7.52	126.60	-0.44 (P=0.02)	0.19	0.17	1.78

Figure 5.112: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

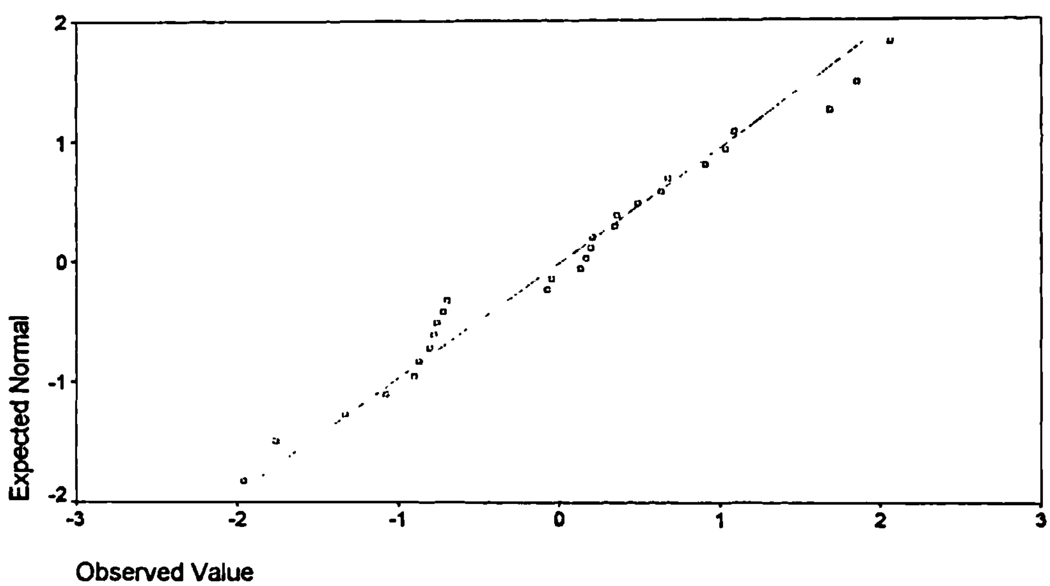


Figure 5.113: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

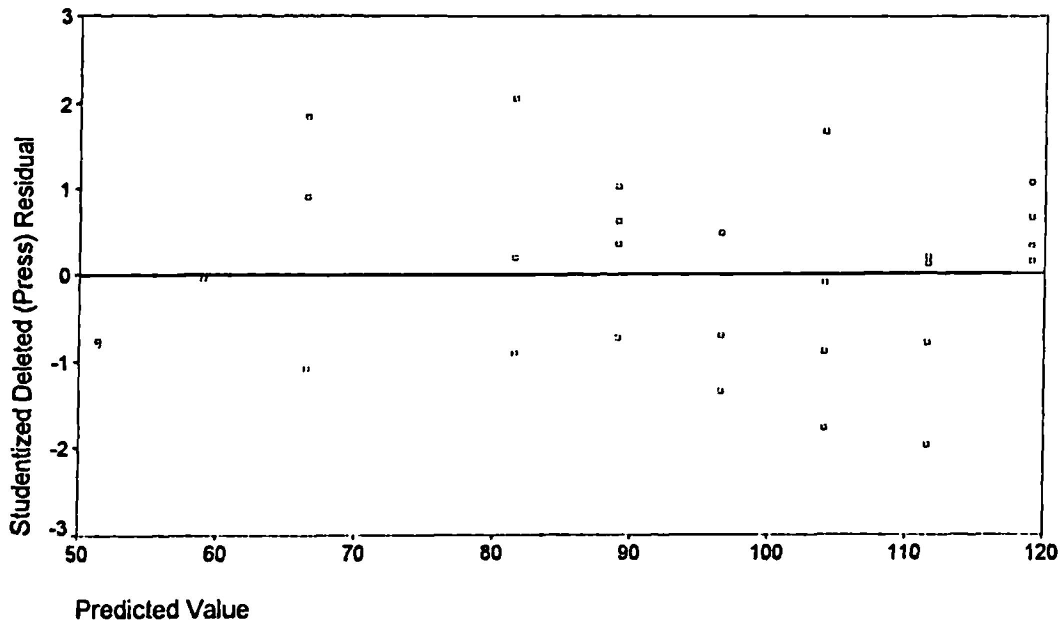
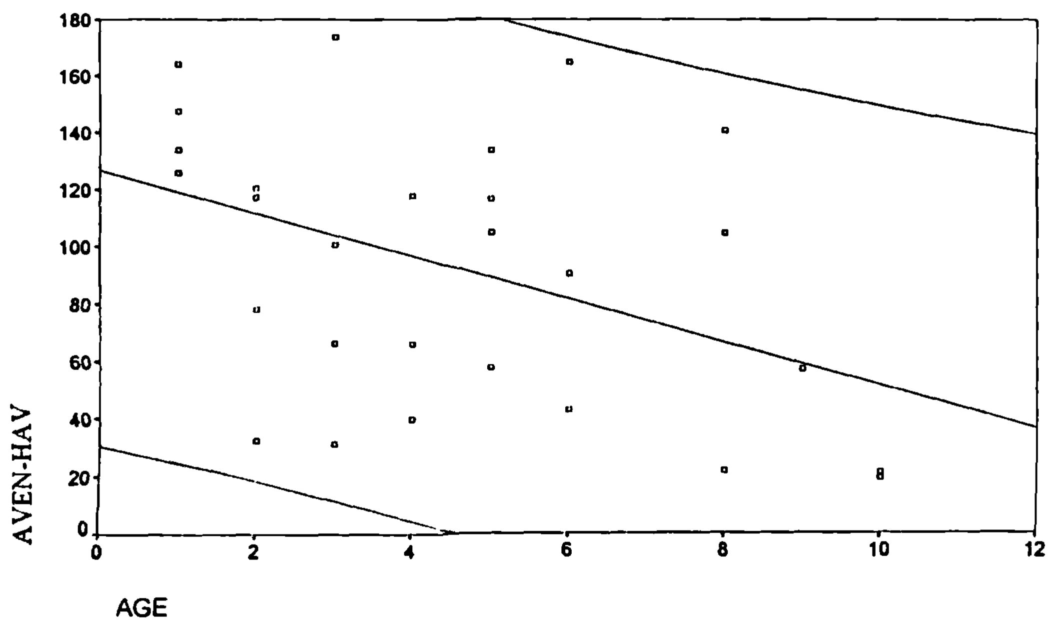


Figure 5.114: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Average Non-Haversian Canal Count (AVEOST) And Cement Layer Age



5.3.2 THE DOES

Regression of secondary osteon count in position 1 on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.32) and the majority of points in the Q-Q plot (Figure 5.115) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.116). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.79 (Table 5.32). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.47 (P=0.00) (Table 5.32). This indicated that there was a highly significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.22 (Table 5.32). This indicated that the model did not fit the relationship between the two variables well and, that, only 22% of a change in secondary osteon count in position 1 could be accounted for by a change in cement layer age.

Table 5.32: Regression Of Secondary Osteon Count In Position 1 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
3.30	16.38	0.47 (P=0.00)	0.22	>0.20	1.79

Figure 5.115: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

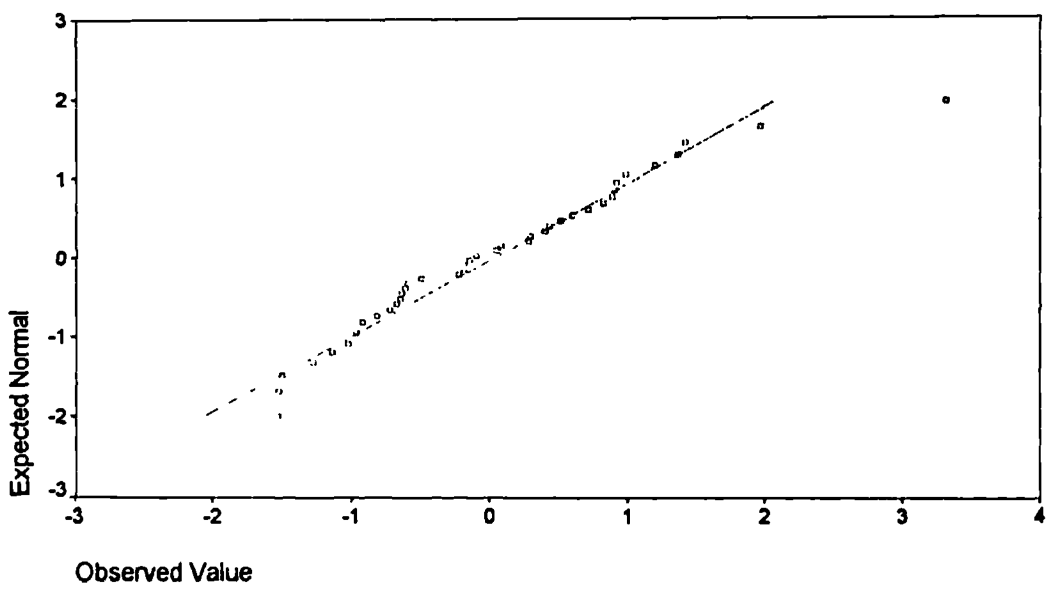


Figure 5.116: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

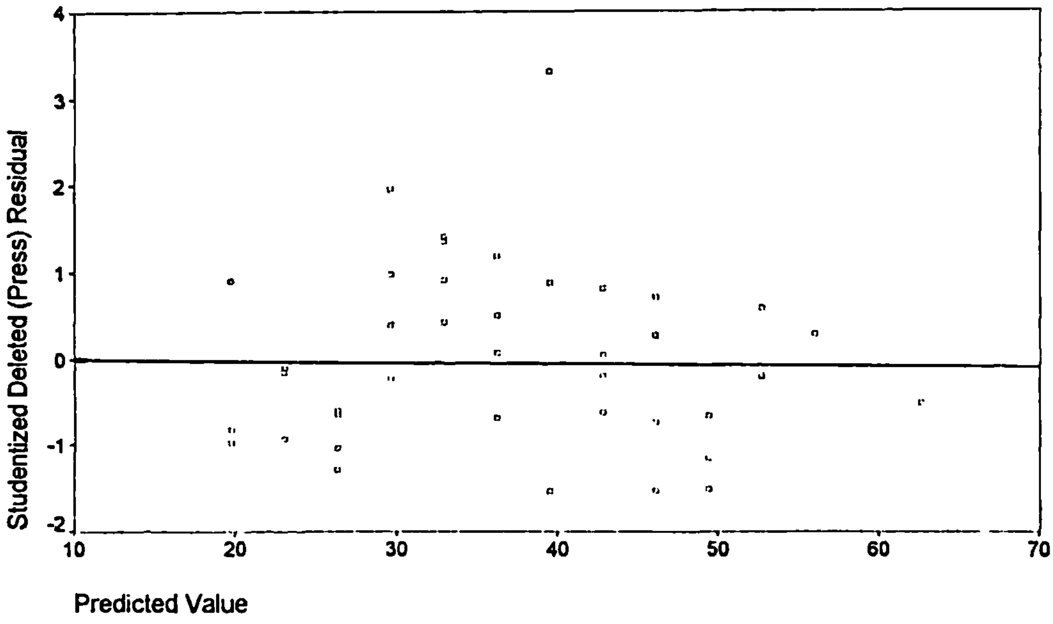
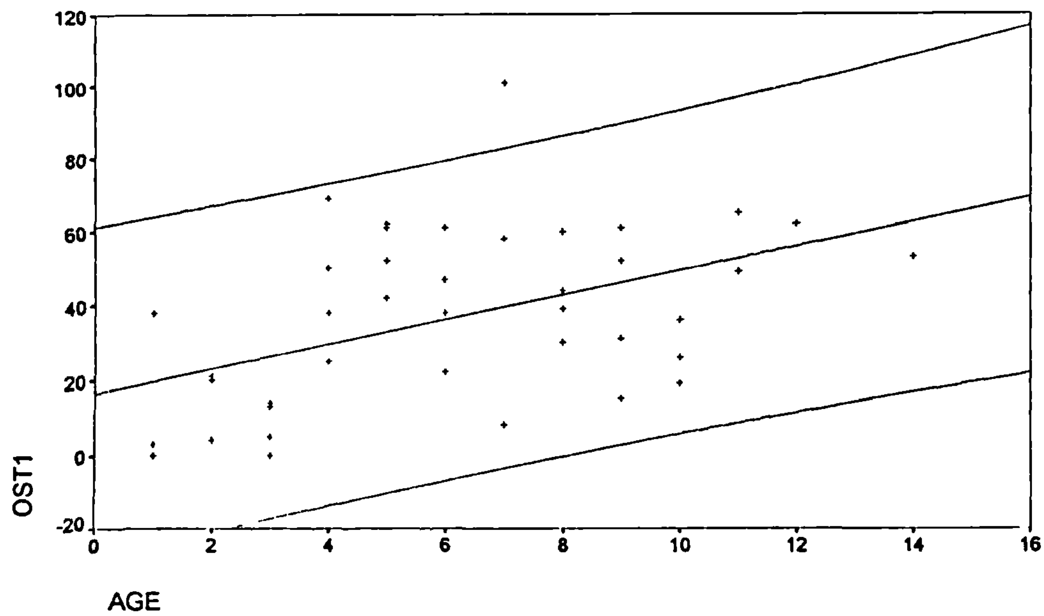


Figure 5.117: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 1 (OST1) And Cement Layer Age



Regression of secondary osteon count in position 2 on cement layer age (n=42):

- The K-S (Lilliefors) significance was 0.05 (Table 5.33) and the majority of points in the Q-Q plot (Figure 5.118) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.119). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.81 (Table 5.33). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.50 (P=0.00) (Table 5.33). This indicated that there was a highly significant, weak degree of association between the two variables.
- The value of r^2 was 25% (Table 5.33). This indicated that the model did not fit the relationship between the two variables well and, that, only 25% of a change in secondary osteon count in position 2 could be accounted for by a change in cement layer age.

Table 5.33: Regression Of Secondary Osteon Count In Position 2 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
4.72	17.26	0.50 (P=0.00)	0.25	0.05	1.81

Figure 5.118: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

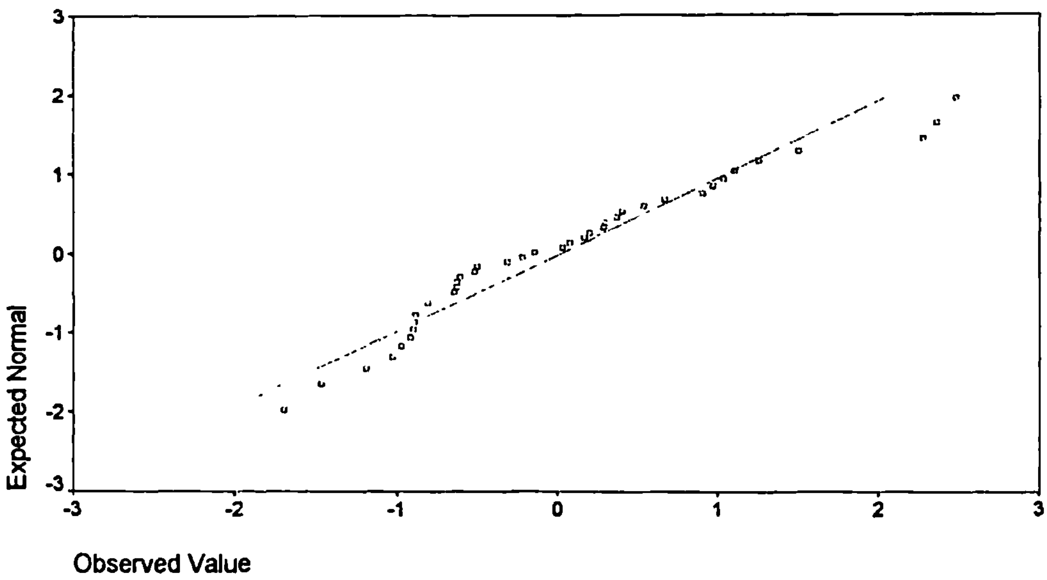


Figure 5.119: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

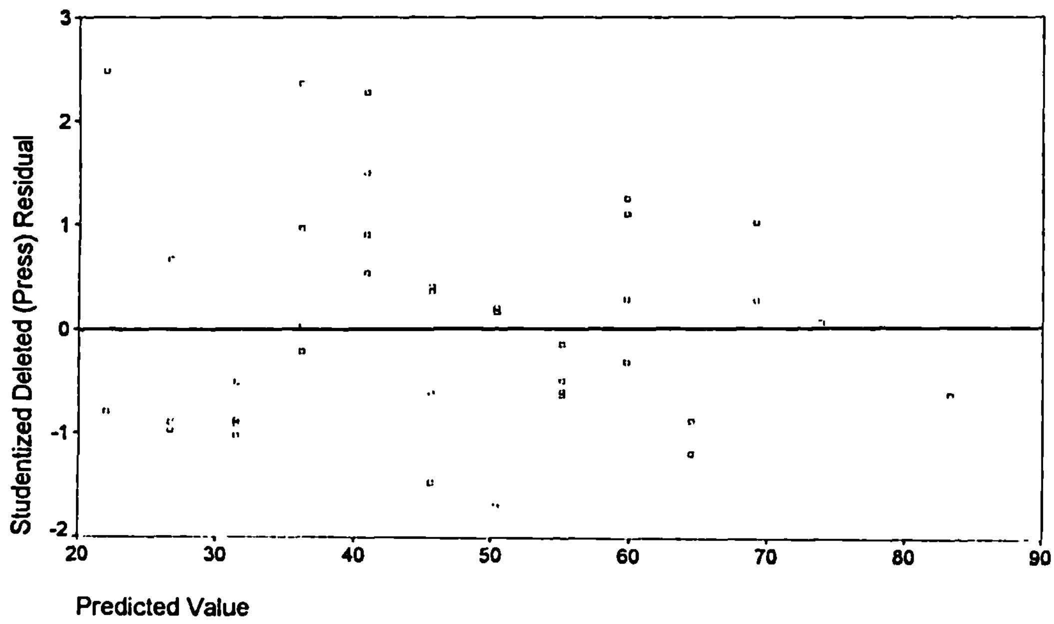
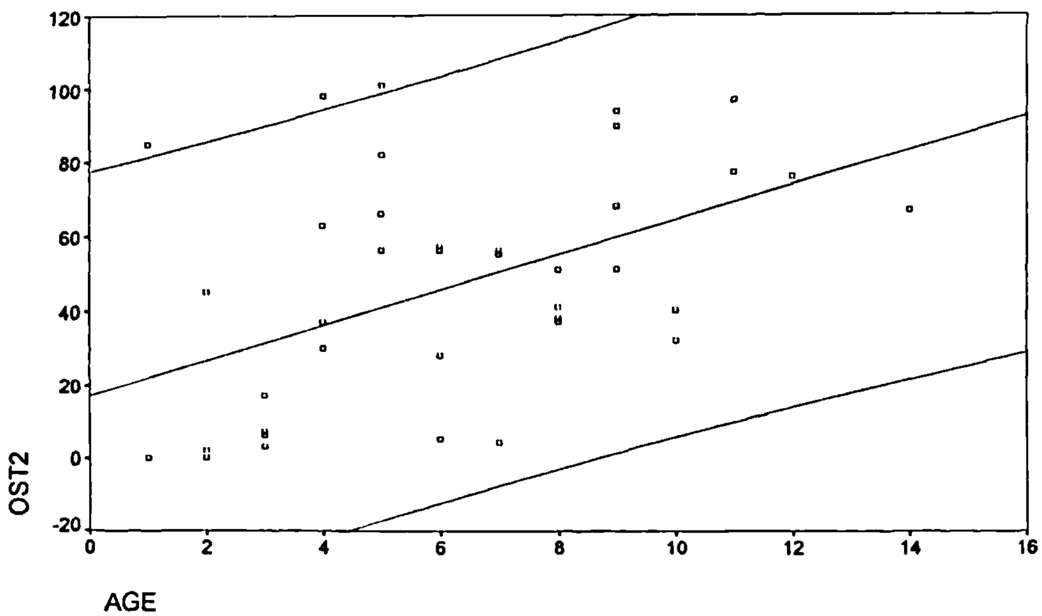


Figure 5.120: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 2 (OST2) And Cement Layer Age



Regression of secondary osteon count in position 3 on cement layer age (n=42):

- The K-S (Lilliefors) significance was 0.16 (Table 5.34) and the majority of points in the Q-Q plot (Figure 5.121) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.122). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.13 (Table 5.34). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.62 (P=0.00) (Table 5.34). This indicated that there was a highly significant, moderate degree of linear association between the two variables.
- The value of r^2 was 0.38 (Table 5.34). This indicated that the model did not fit the relationship between the two variables very well and, that, only 38% of a change in secondary osteon count in position 3 could be accounted for by a change in cement layer age.

Table 5.34: Regression Of Secondary Osteon Count In Position 3 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
4.74	6.67	0.62 (P=0.00)	0.38	0.16	2.13

Figure 5.121: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

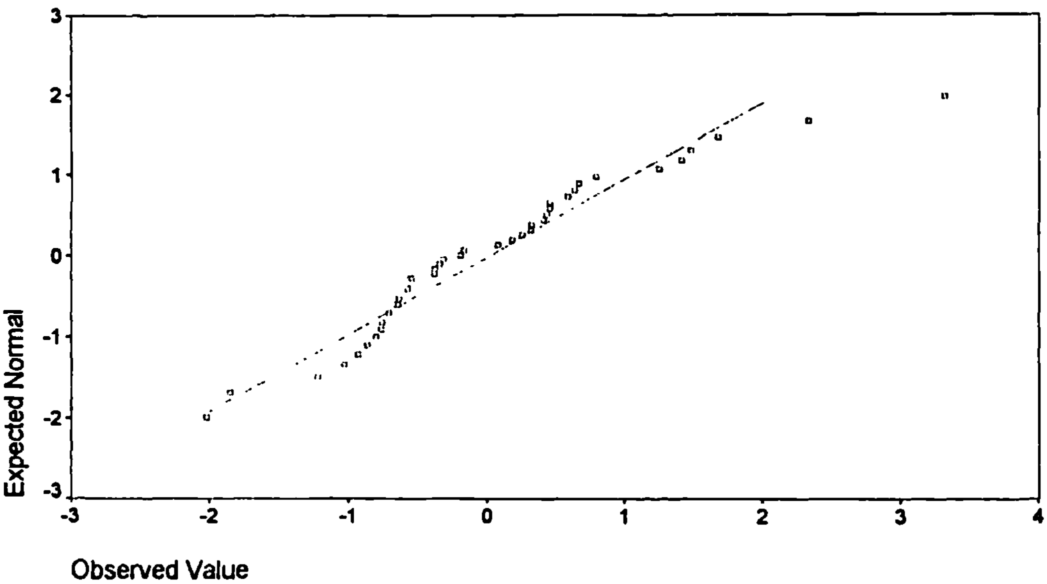


Figure 5.122: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

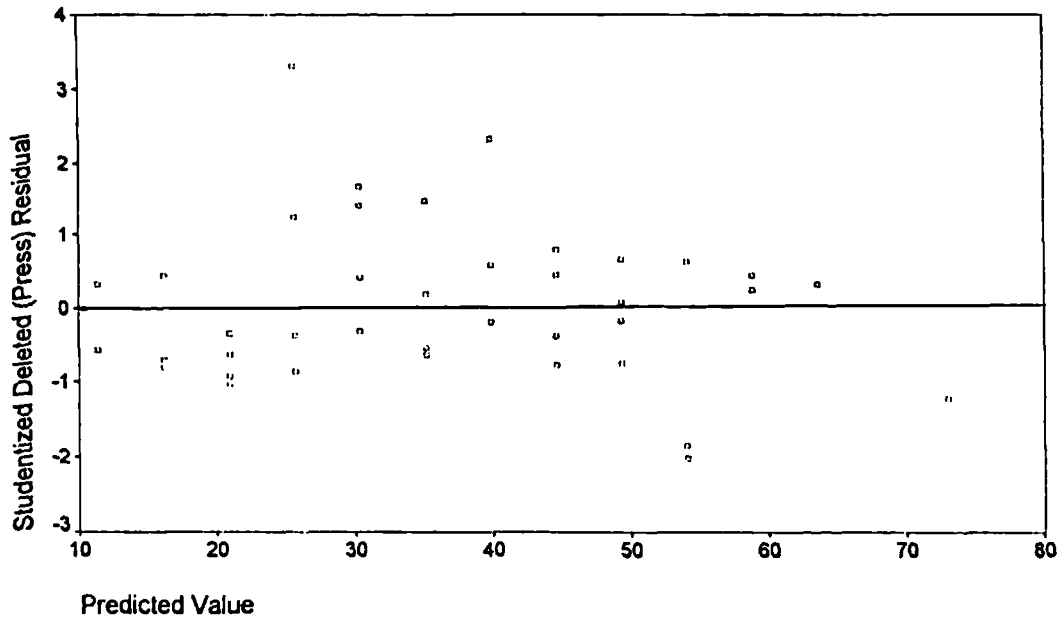
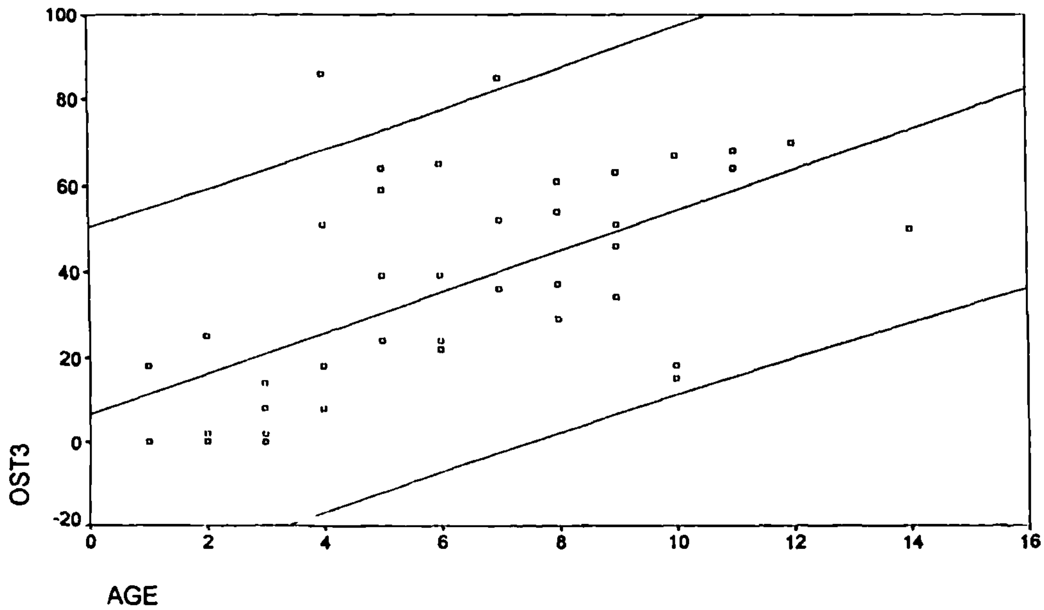


Figure 5.123: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 3 (OST3) And Cement Layer Age



Regression of secondary osteon count in position 4 on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.35) and the majority of points in the Q-Q plot (Figure 5.124) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.125). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.25 (Table 5.35). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.59 (P=0.00) (Table 5.35). This indicated that there was a highly significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.35 (Table 5.35). This indicated that the model did not fit the relationship between the two variables well and, that, only 35% of a change in secondary osteon count in position 4 could be accounted for by a change in cement layer age.

Table 5.35: Regression Of Secondary Osteon Count In Position 4 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
3.95	15.66	0.59 (P=0.00)	0.35	>0.20	2.25

Figure 5.124: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

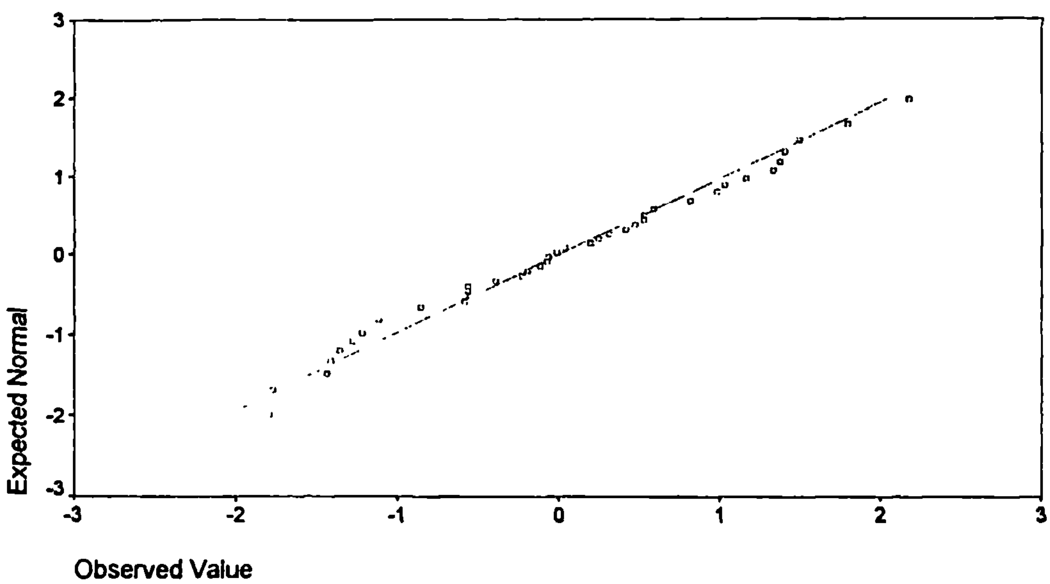


Figure 5.125: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

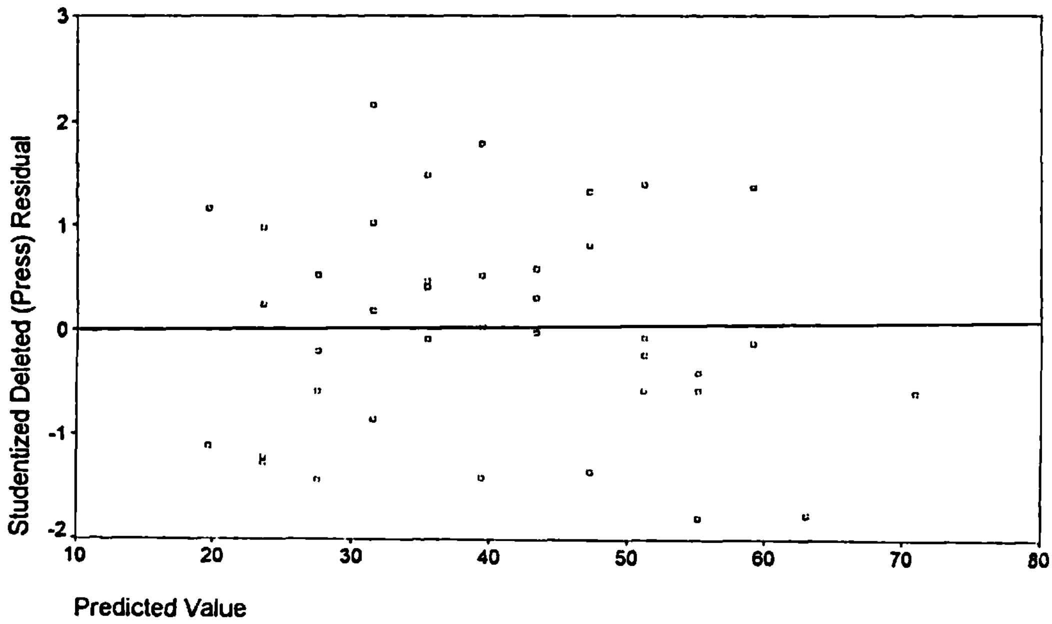
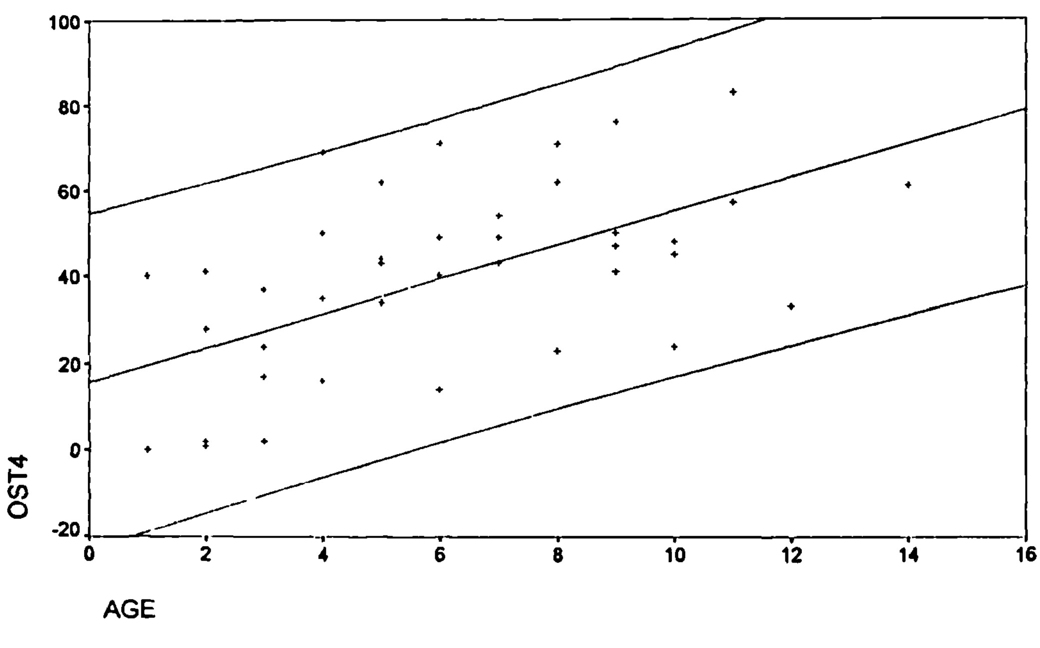


Figure 5.126: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Secondary Osteon Count In Position 4 (OST4) And Cement Layer Age



Regression of total secondary osteon count on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.36) and the majority of points in the Q-Q plot (Figure 5.127) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.128). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.92 (Table 5.36). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.61 (P=0.00) (Table 5.36). This indicated that there was a highly significant, moderate degree of linear association between the two variables.
- The value of r^2 was 0.37 (Table 5.36). This indicated that the model did not fit the relationship between the two variables very well and, that, only 37% of a change in total secondary osteon count could be accounted for by a change in cement layer age.

Table 5.36: Regression Of Total Secondary Osteon Count On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
16.80	56.14	0.61 (P=0.00)	0.37	>0.20	1.92

Figure 5.127: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

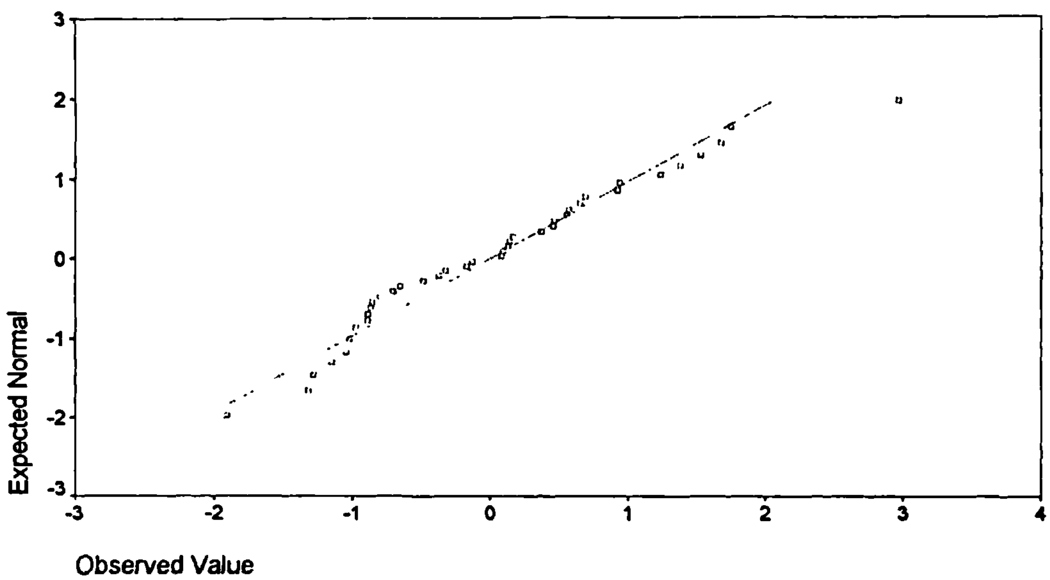


Figure 5.128: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

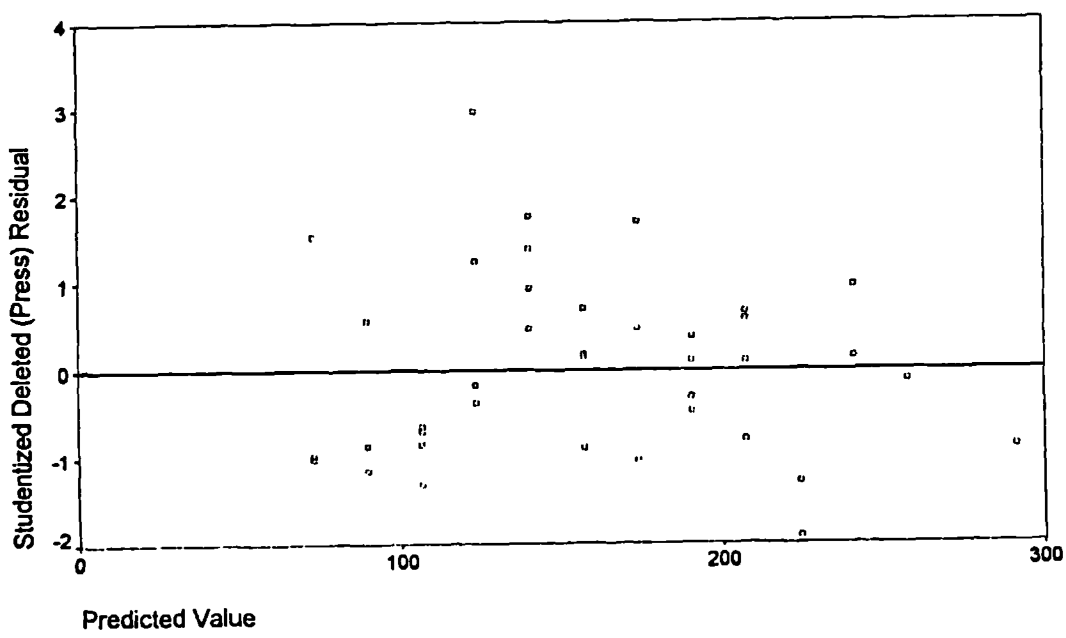
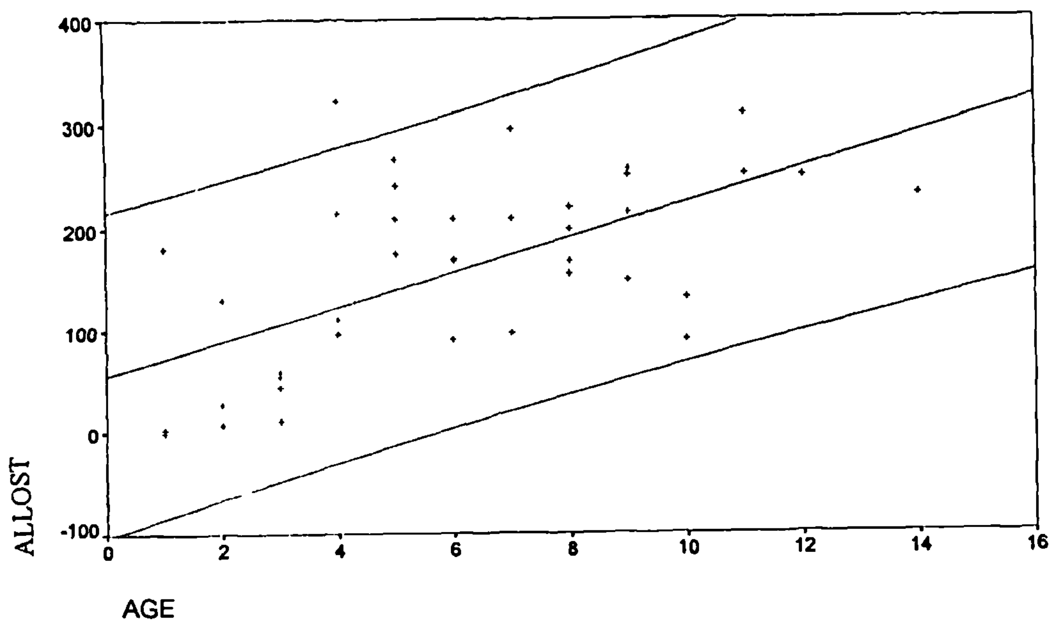


Figure 5.129: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Total Secondary Osteon Count (ALLOST) And Cement Layer Age



Regression of average secondary osteon count on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.37) and that the majority of points in the Q-Q plot (Figure 5.130) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.131). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.91 (Table 5.37). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.61 (P=0.00) (Table 5.37). This indicated that there was a highly significant, moderate degree of linear association between the two variables.
- The value of r^2 was 0.37 (Table 5.37). This indicated that the model did not fit the relationship between the two variables very well and, that, 37% of a change in average secondary osteon count could be accounted for by a change in cement layer age.

Table 5.37: Regression Of Average Secondary Osteon Count On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
4.23	13.77	0.61 (P=0.00)	0.39	>0.20	1.91

Figure 5.130: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

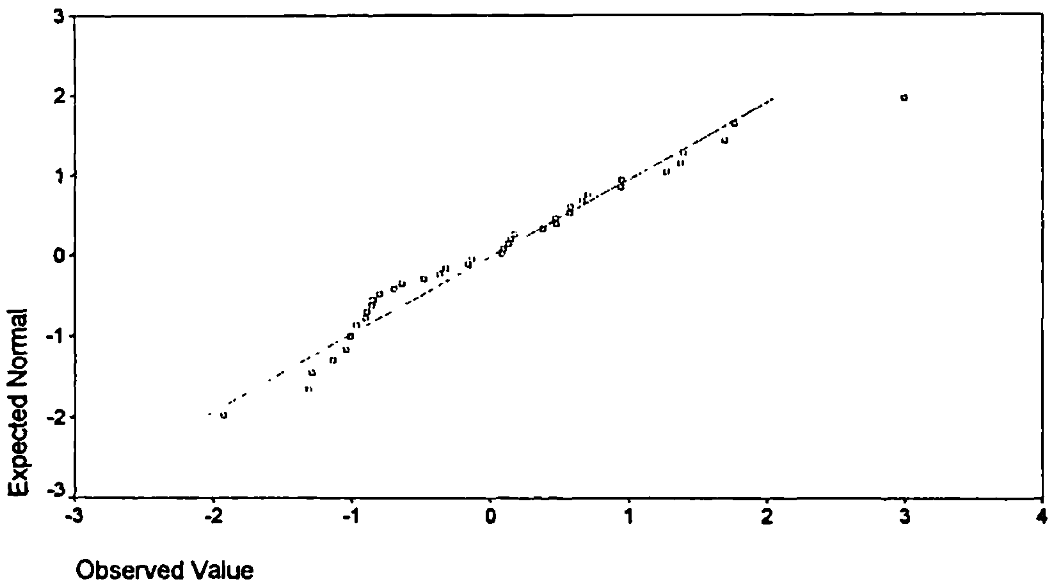


Figure 5.131: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

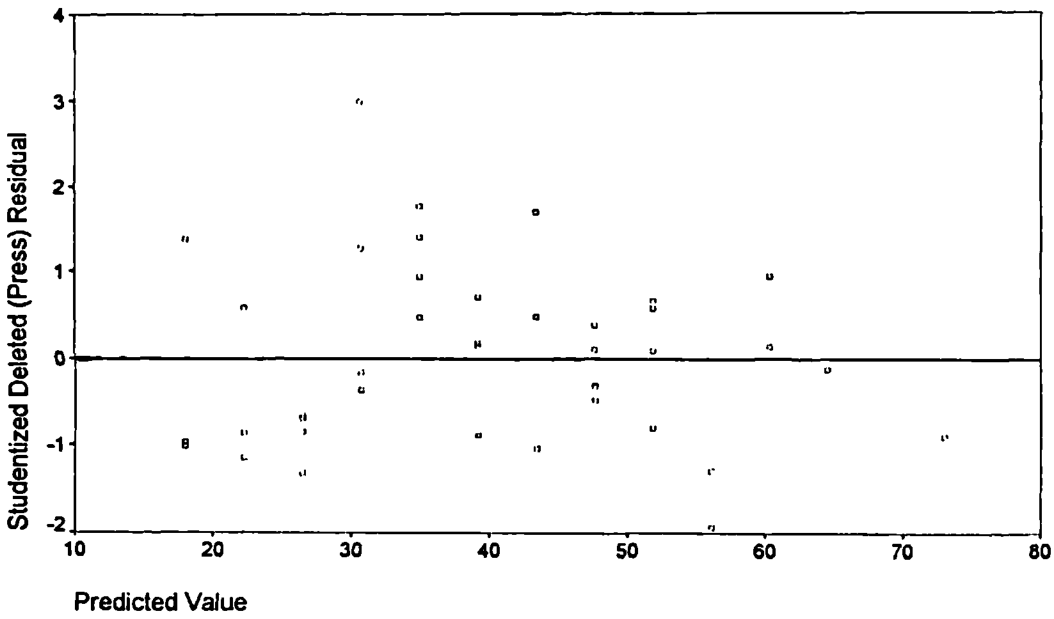
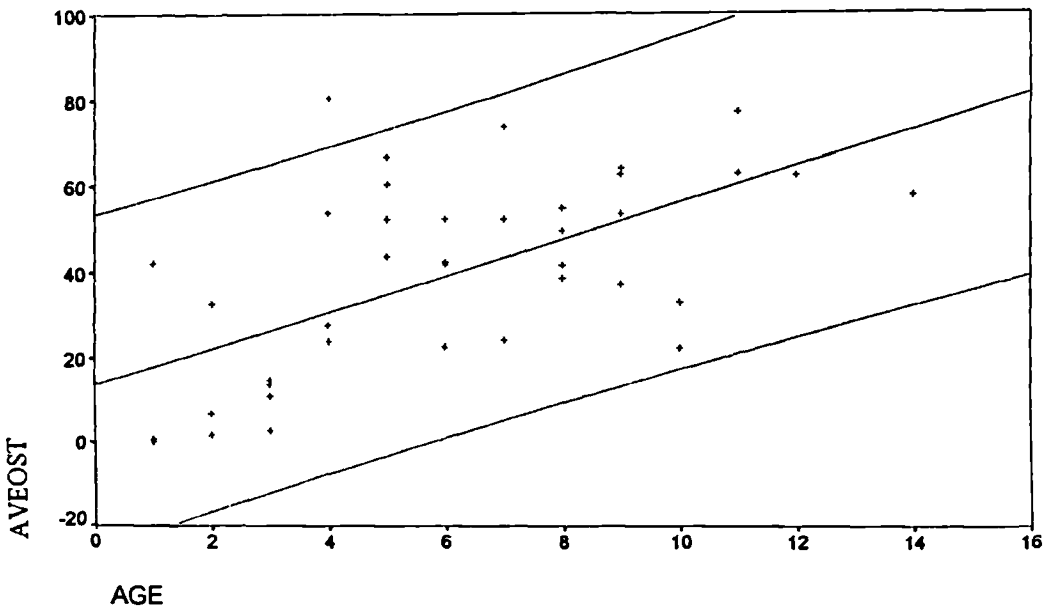


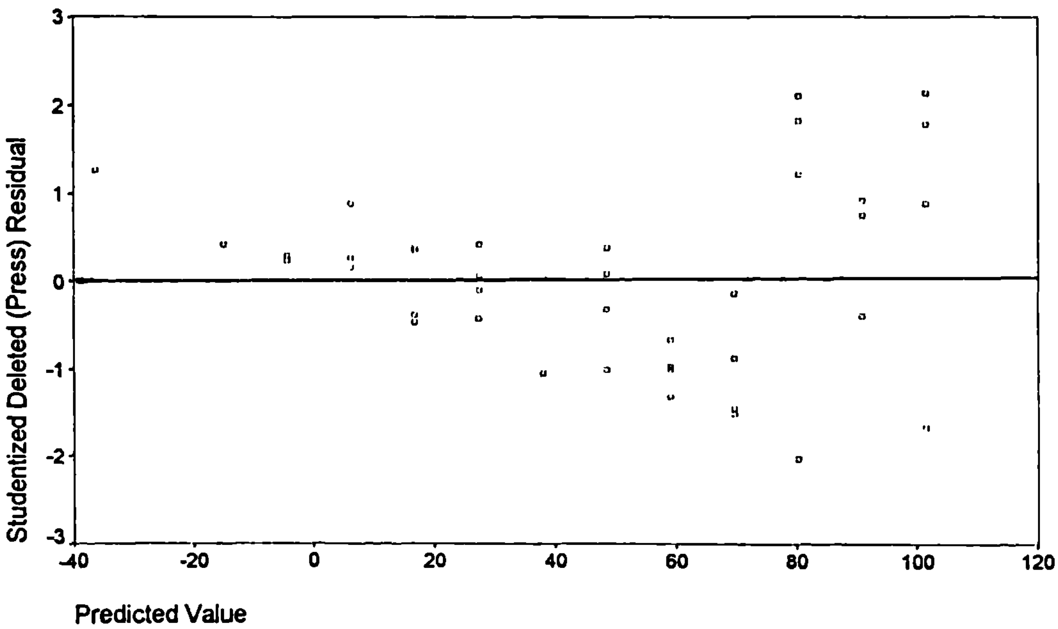
Figure 5.132: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Average Secondary Osteon Count (AVEOST) And Cement Layer Age



Regression of non-Haversian canal count in position 1 on cement layer age (n=42):

- The studentised deleted residuals were distributed around 0 (Figure 5.133), but their distribution was not random, and their variability generally increased with an increase in predicted values. This indicated that they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between transformed non-Haversian canal count in position 1 and cement layer age (below).

Figure 5.133: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 1 on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.38) and the majority of points in the Q-Q plot (Figure 5.134) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.135). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.03 (Table 5.38). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.72 (P=0.00) (Table 5.38). This indicated that there was a highly significant, strong degree of linear association between the two variables.
- The value of r^2 was 0.51 (Table 5.38). This indicated that the model fitted the data quite well and, that, 42% of a change in transformed non-Haversian canal count in position 1 could be accounted for by a change in cement layer age.

Table 5.38: Regression Of Transformed Non-Haversian Canal Count In Position 1 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.79	10.63	− 0.72 (P=0.00)	0.51	>0.20	2.03

Figure 5.134: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

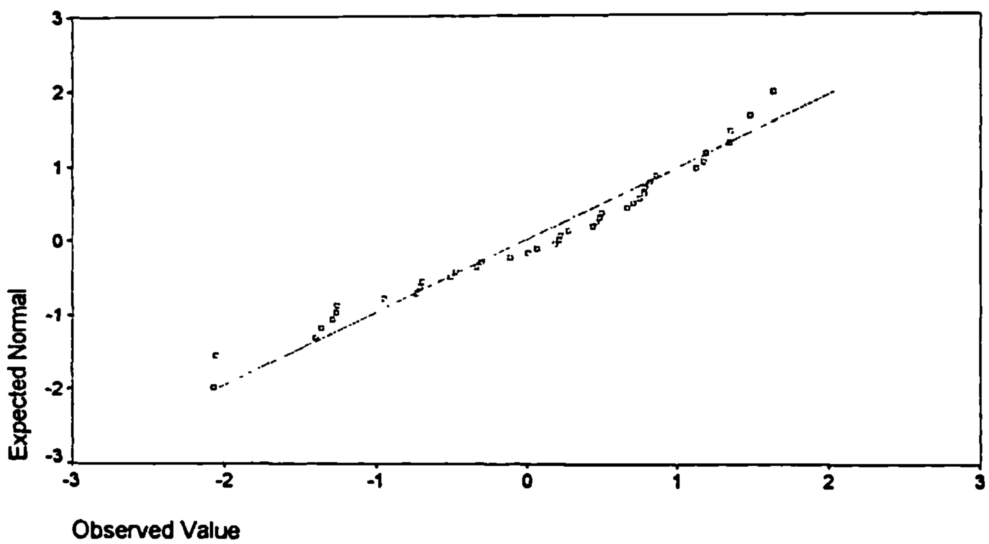


Figure 5.135: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

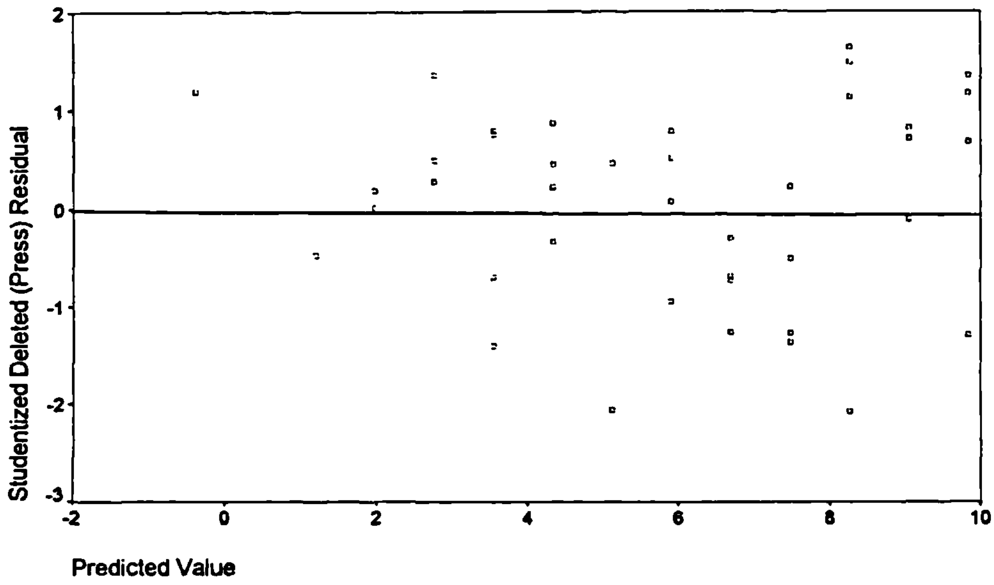
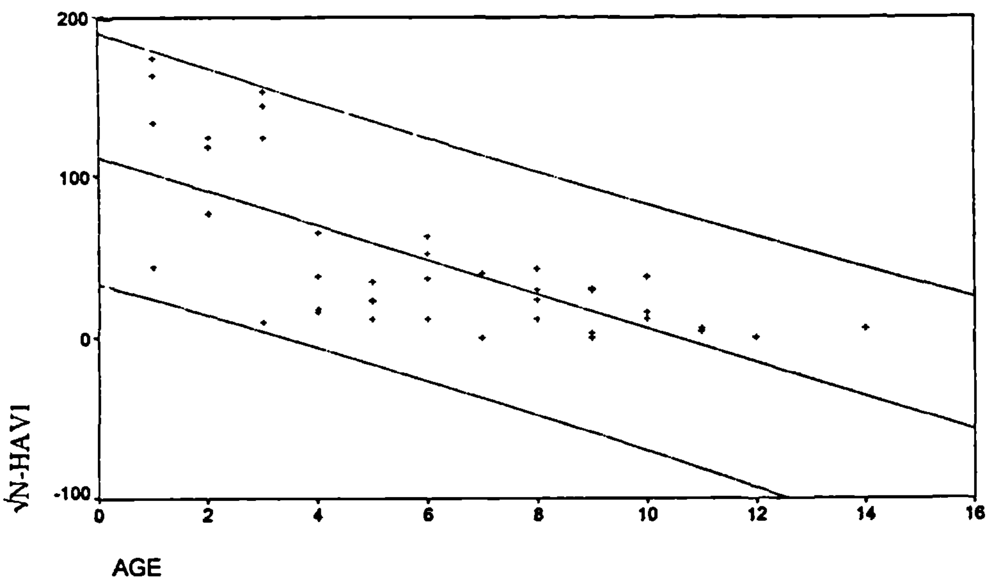


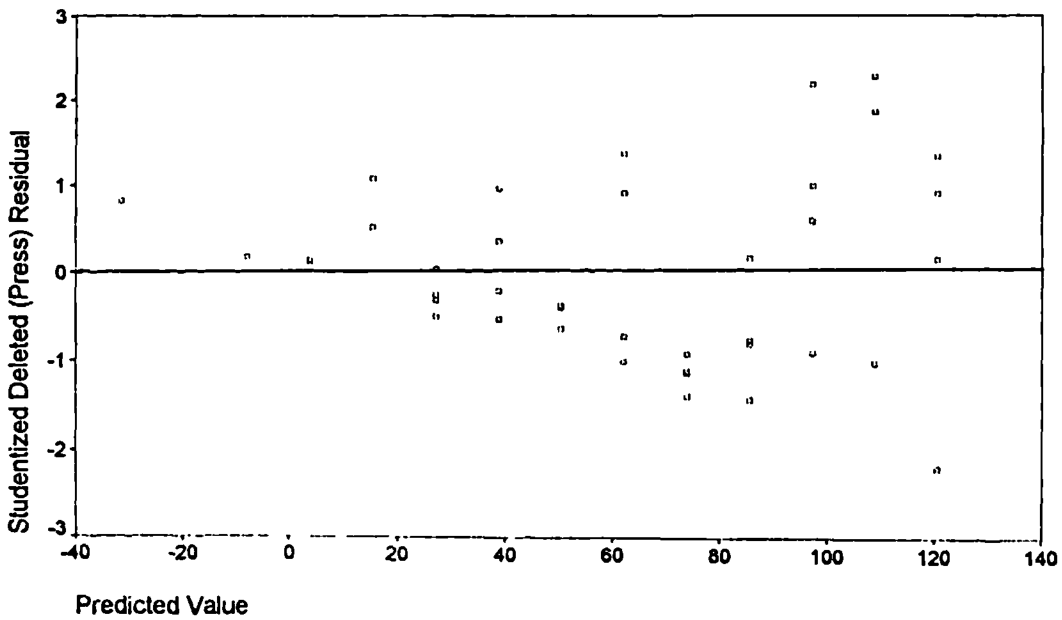
Figure 5.136: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 1 (√N-HAV1) And Cement Layer Age



Regression of non-Haversian canal count in position 2 on cement layer age (n=42):

- The studentised deleted residuals were distributed around 0 (Figure 5.137), but their distribution was not random, and their variability generally increased with an increase in predicted values. This indicated that they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new linear regression model was fitted to the relationship between transformed non-Haversian canal count in position 2 and cement layer age (below).

Figure 5.137: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 2 on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.39) and the majority of points in the Q-Q plot (Figure 5.138) fell along a straight line. This indicated that the studentised deleted residuals had normal distribution.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.139). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.30 (Table 5.39). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.59 (P=0.00) (Table 5.39). This indicated that there was a highly significant, weak degree of linear association between the two variables.
- The value of r^2 was 0.35 (Table 5.39). This indicated that the model did not fit the relationship between the two variables well and, that, 35% of a change in transformed non-Haversian canal count in position 2 could be accounted for by a change in cement layer age.

Table 5.39: Regression Of Transformed Non-Haversian Canal Count In Position 2 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.73	11.10	− 0.59 (P=0.00)	0.35	>0.20	2.30

Figure 5.138: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

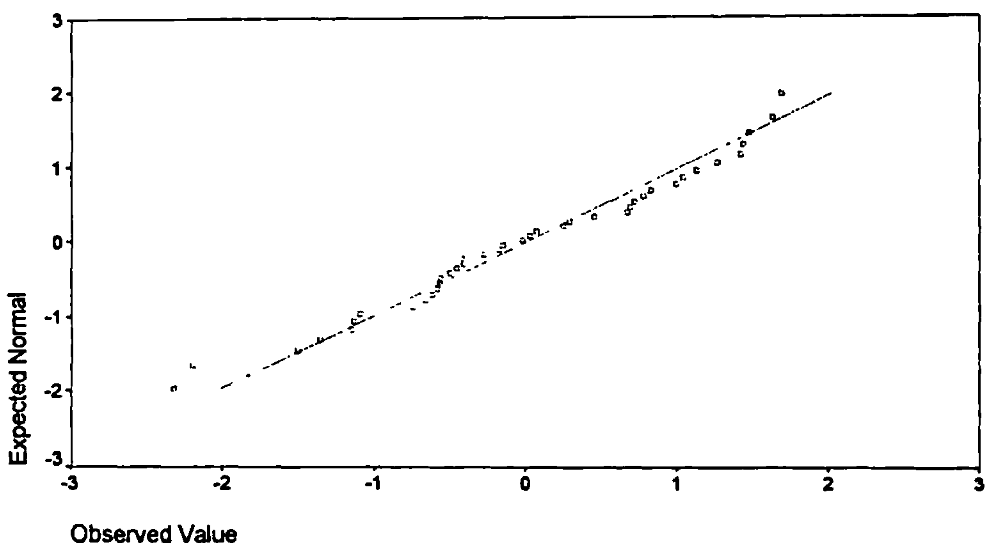


Figure 5.139: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

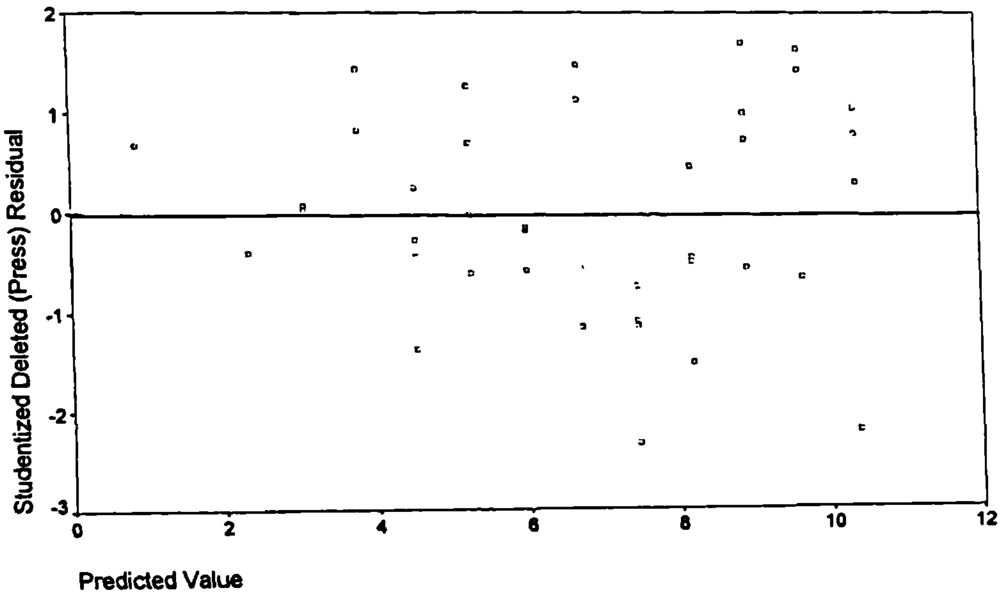
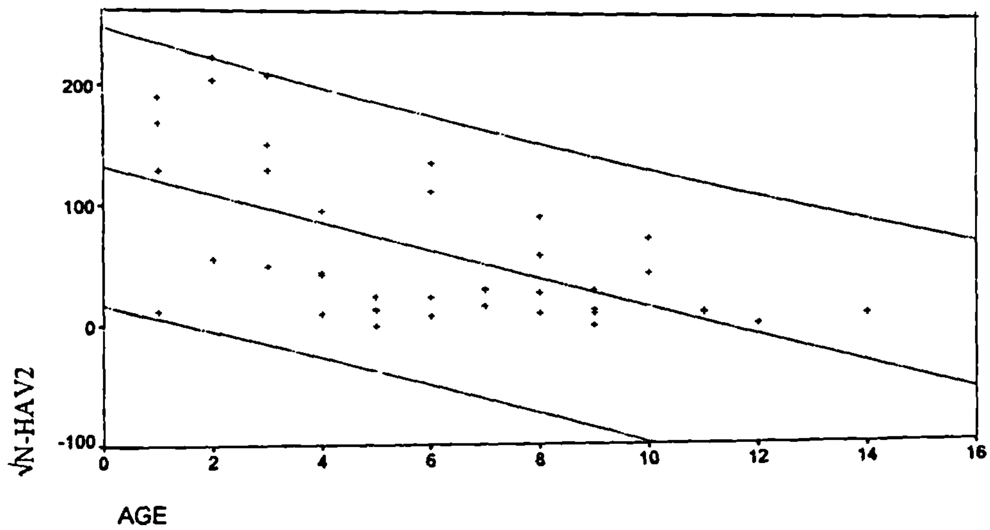


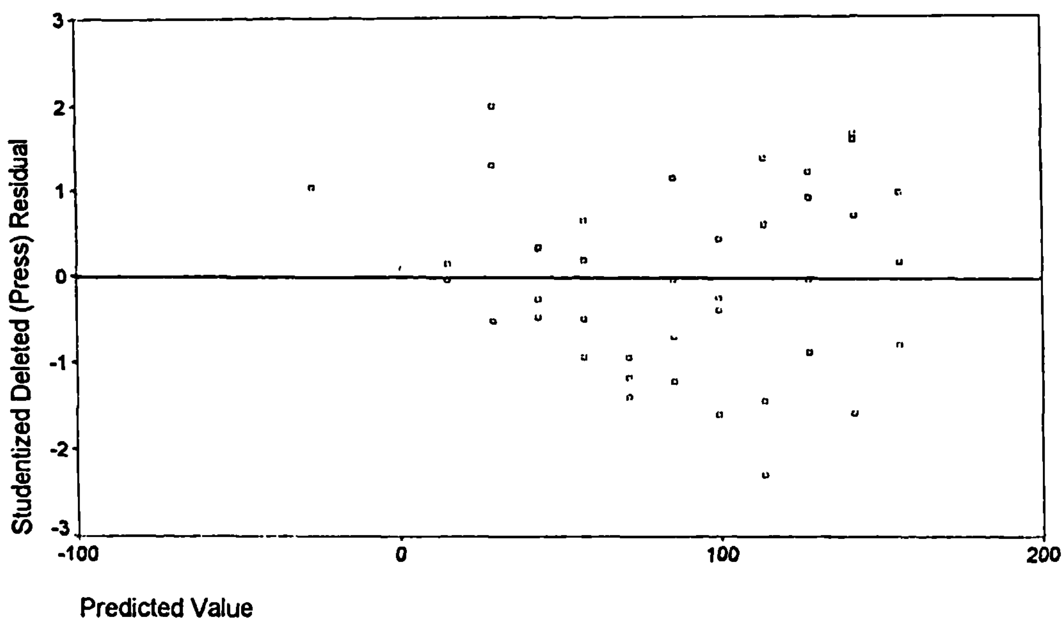
Figure 5.140: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 2 (√N-HAV2) And Cement Layer Age



Regression of non-Haversian canal count in position 3 on cement layer age (n=42):

- The studentised deleted residuals were distributed around 0 (Figure 5.141), but their distribution was not random, and their variability increased with an increase in predicted values. This indicated that they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new model was fitted to the relationship between transformed non-Haversian canal count in position 3 and cement layer age (below).

Figure 5.141: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 3 on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.40) and the majority of points in the Q-Q plot (Figure 5.142) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.143). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.37 (Table 5.40). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.70 (P=0.00) (Table 5.40). This indicated that there was a highly significant, strong degree of linear association between the two variables.
- The value of r^2 was 0.48 (Table 5.40). This indicated that the model fitted the relationship between the two variables well and, that, 48% of a change in transformed non-Haversian canal could be accounted for by a change in cement layer age.

Table 5.40: Regression Of Transformed Non-Haversian Canal Count In Position 3 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.81	13.22	− 0.70 (P=0.00)	0.48	>0.20	2.37

Figure 5.142: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

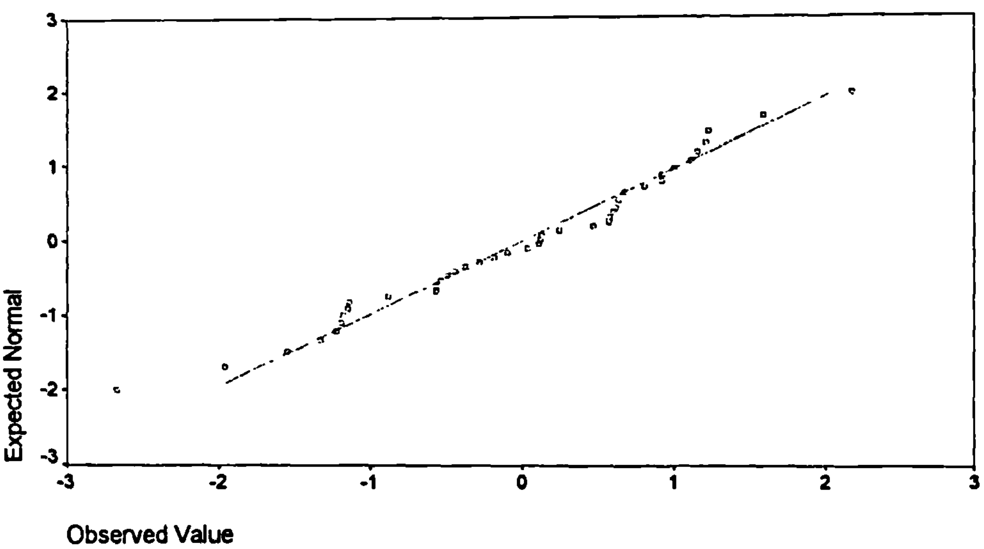


Figure 5.143: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

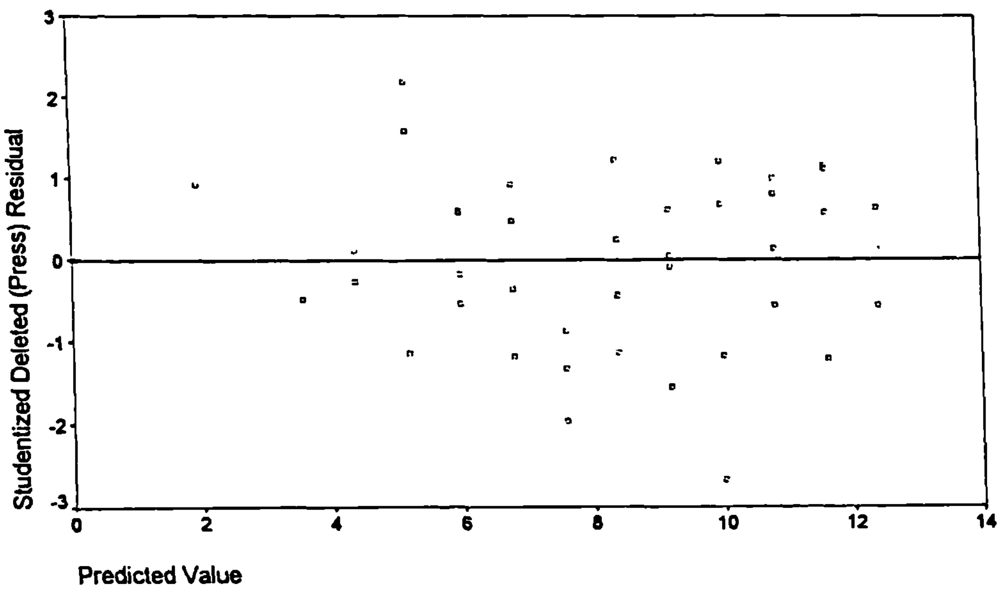
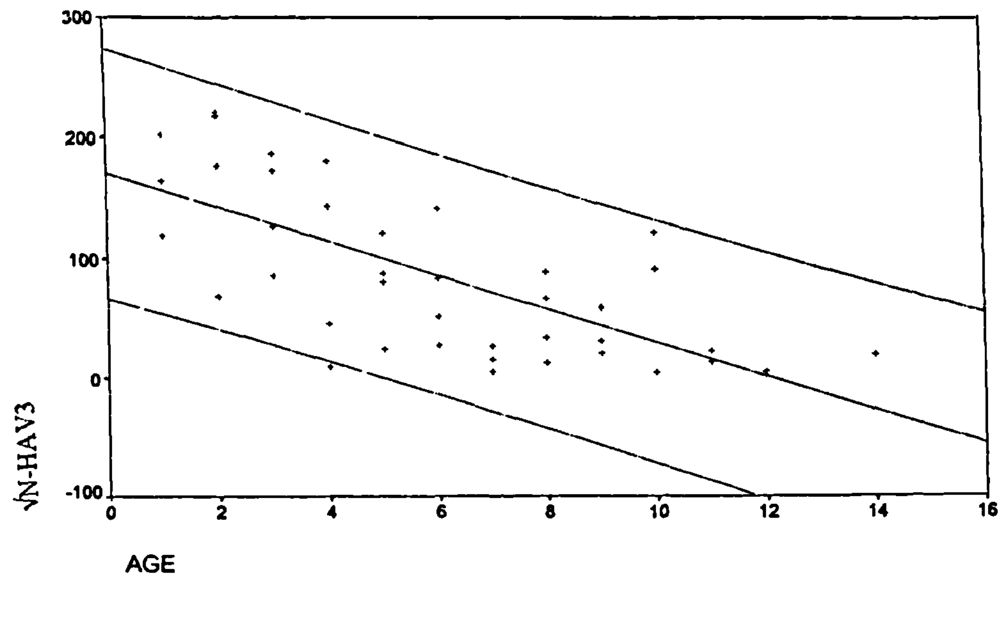


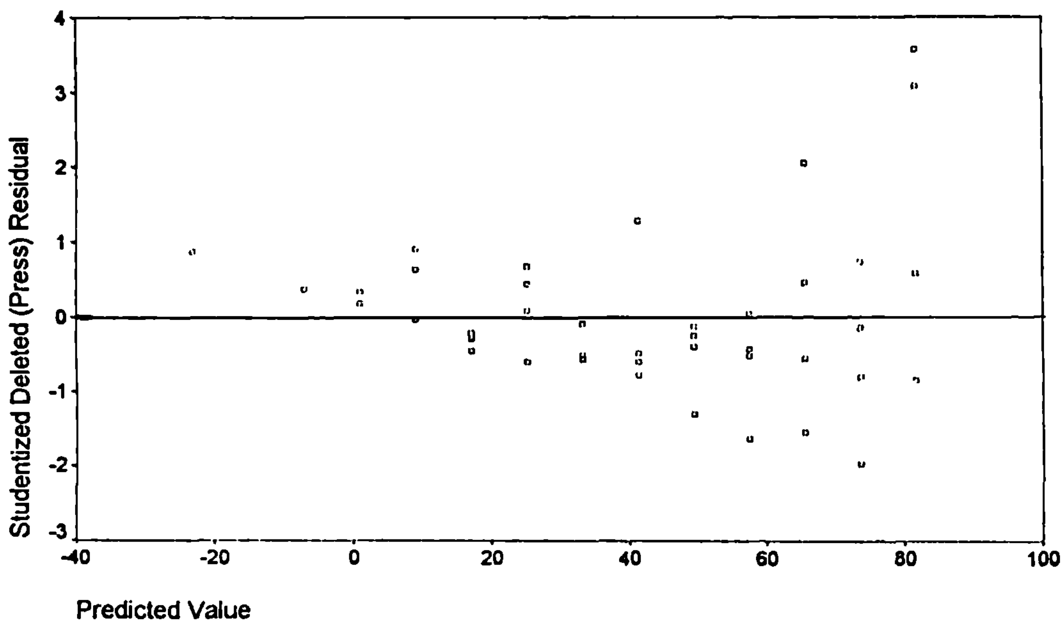
Figure 5.144: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 3 (√N-HAV3) And Cement Layer Age



Regression of non-Haversian canal count in position 4 on cement layer age (n=42):

- The studentised deleted residuals were distributed around 0 (Figure 5.145), but their distribution was not random, and their variability increased with an increase in predicted value. This indicated that they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new model was fitted to the relationship between transformed non-Haversian canal count in position 4 and cement layer age (below).

Figure 5.145: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed non-Haversian canal count in position 4 on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.41) and the majority of points in the Q-Q plot (Figure 5.146) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.147). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.90 (Table 5.41). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.68 (P=0.00) (Table 5.41). This indicated that there was a highly significant, moderate degree of association between the two variables.
- The value of r^2 was 0.46 (Table 5.41). This indicated that the model fitted the relationship between the two variables quite well and, that, 46% of a change in transformed non-Haversian canal count in position 4 could be accounted for by a change in cement layer age.

Table 5.41: Regression Of Transformed Non-Haversian Canal Count In Position 4 On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.60	9.31	-0.68 (P=0.00)	0.46	>0.20	1.90

Figure 5.146: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

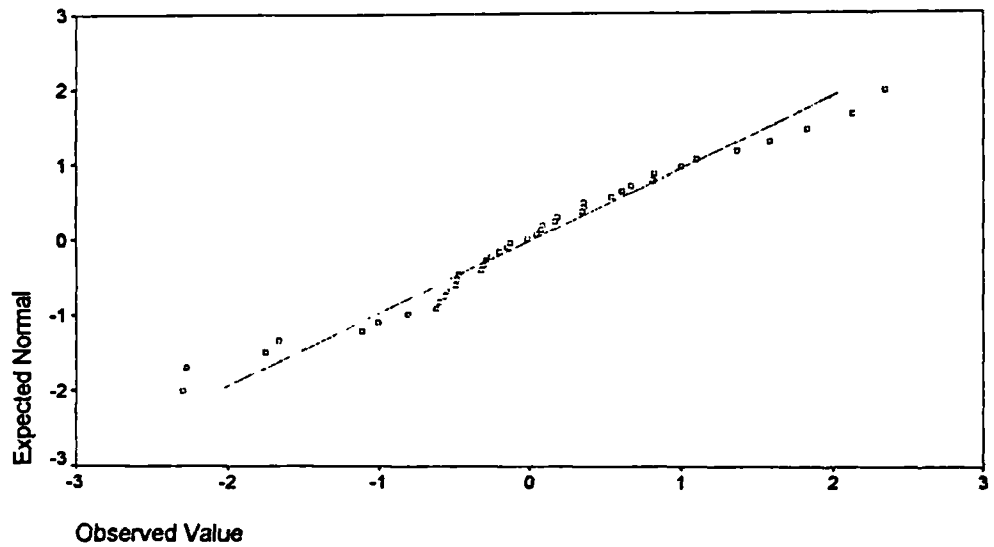


Figure 5.147: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

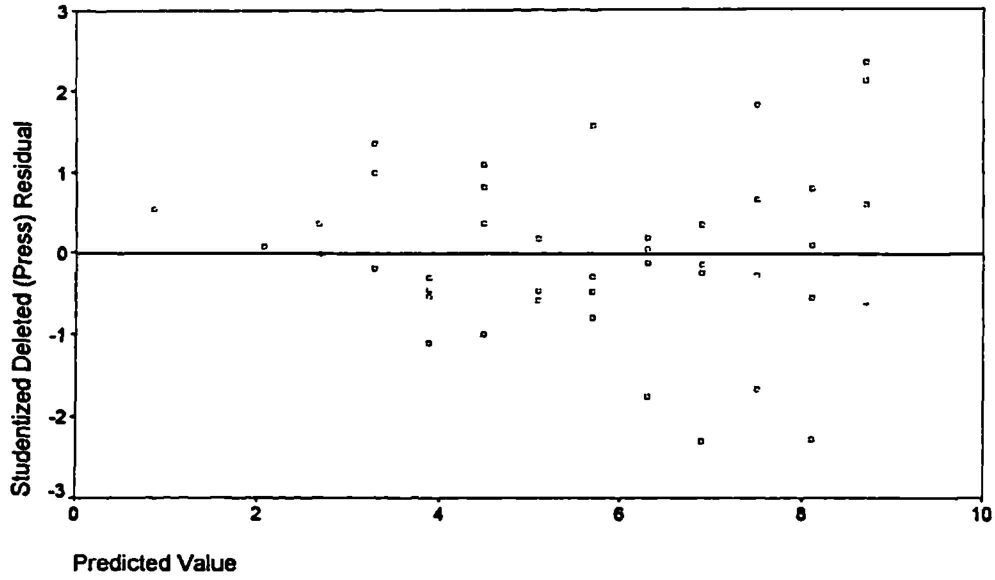
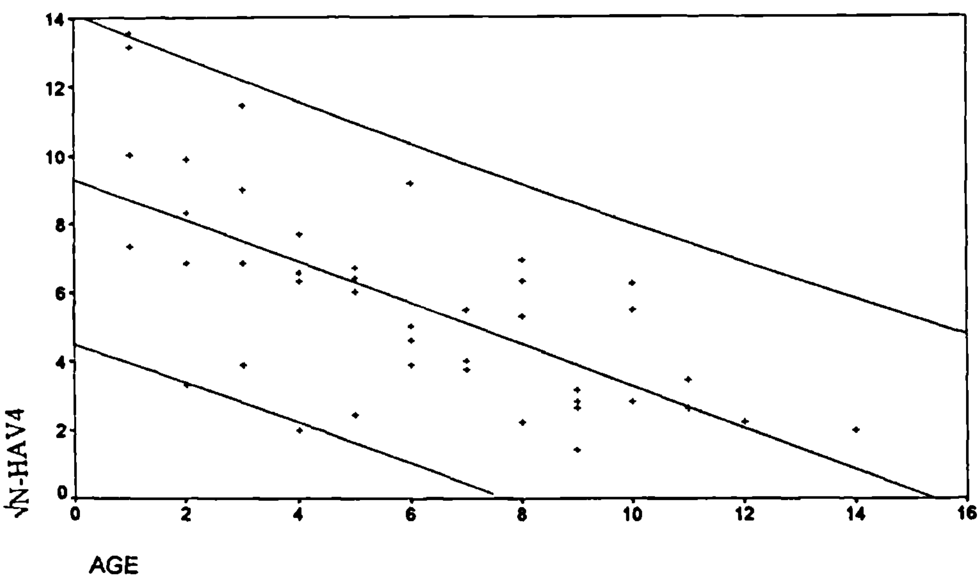


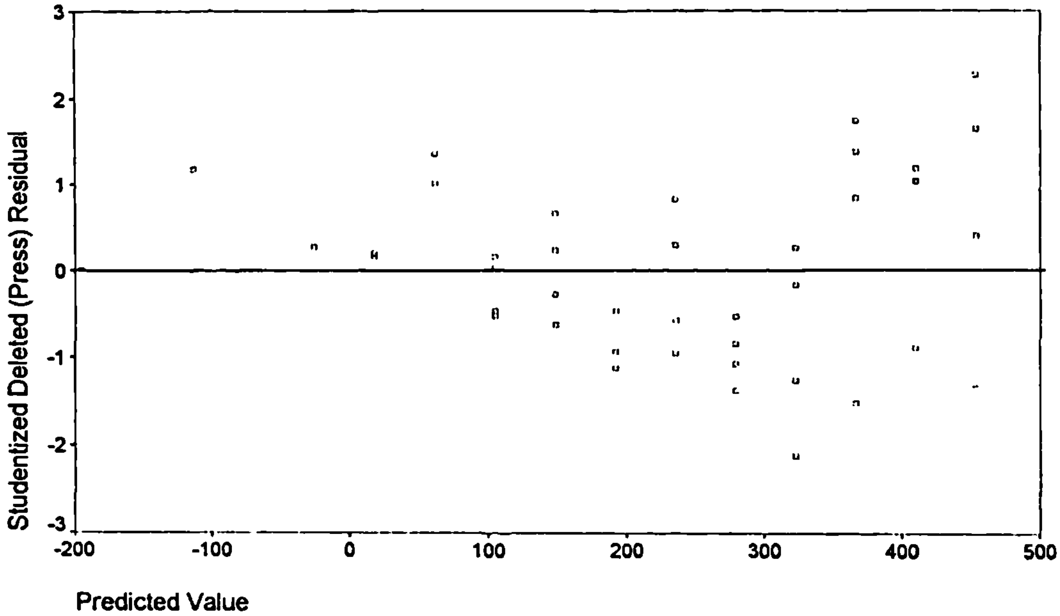
Figure 5.148: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Non-Haversian Canal Count In Position 4 (√N-HAV4) And Cement Layer Age



Regression of total non-Haversian canal count on cement layer age (n=42):

- The studentised deleted residuals were distributed around 0 (Figure 5.149), but their distribution was not random, and their variability increased with an increase in predicted value. This indicated that they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new model was fitted to the relationship between total non-Haversian canal count and cement layer age (below).

Figure 5.149: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed total non-Haversian canal count on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.4.2) and the majority of points in the Q-Q plot (Figure 5.150) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.151). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.40 (Table 5.42). This indicated that the studentised deleted residuals were normally distributed.
- The Pearson's correlation coefficient was -0.75 (P=0.00) (Table 5.42). This indicated that there was a highly significant, strong degree of linear association between the two variables.
- The value of r^2 was 0.56 (Table 5.42). This indicated that the model fitted the data well and, that, 56% of a change in transformed total non-Haversian canal count could be accounted for by a change in cement layer age.

Table 5.42: Regression Of Transformed Total Non-Haversian Canal Count On Cement Layer Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-1.45	22.60	− 0.75 (P=0.00)	0.56	>0.20	2.40

Figure 5.150: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

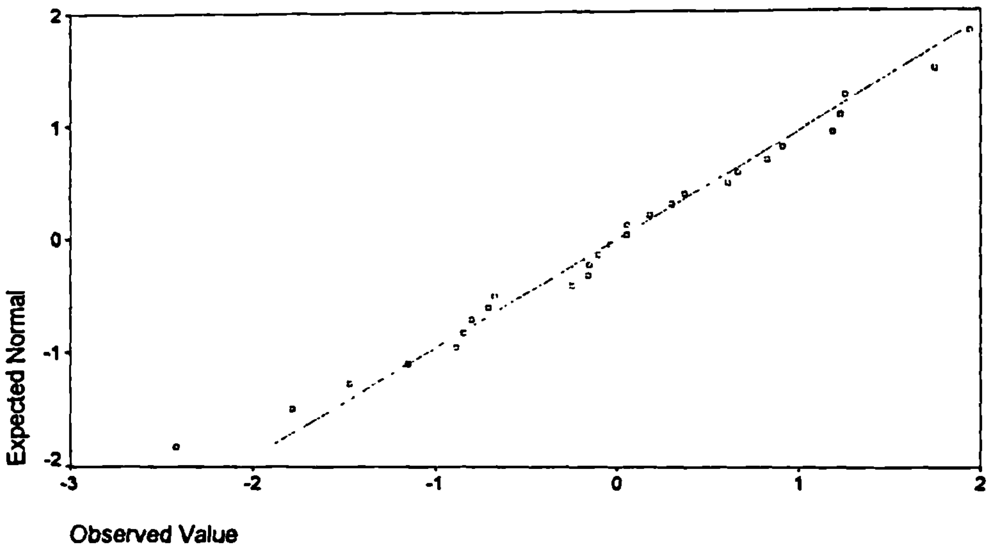


Figure 5.151: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

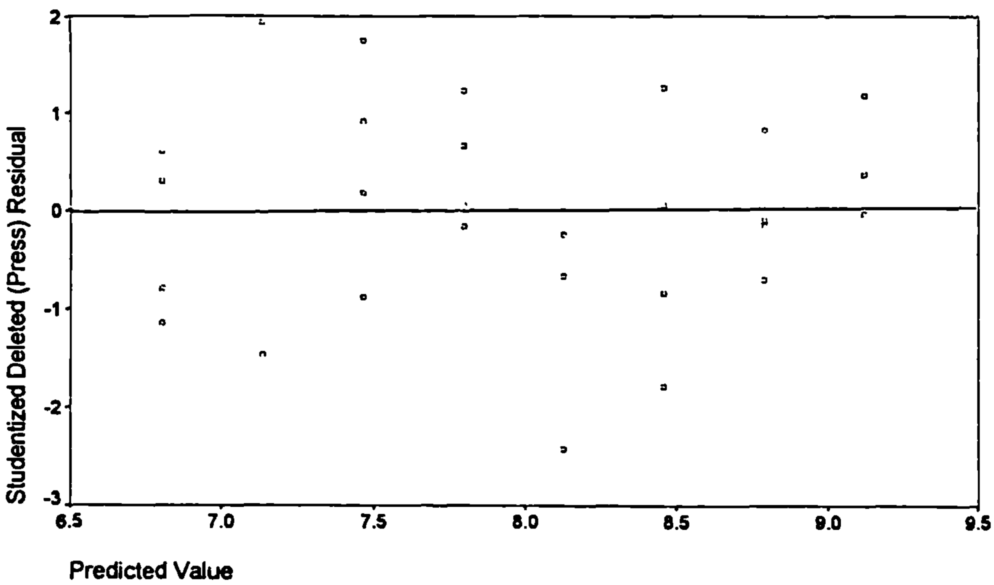
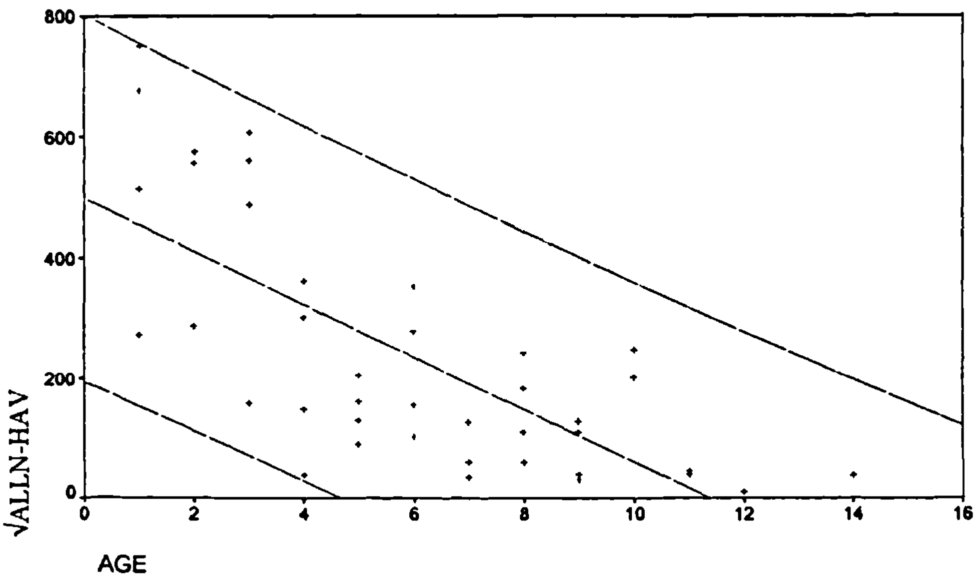


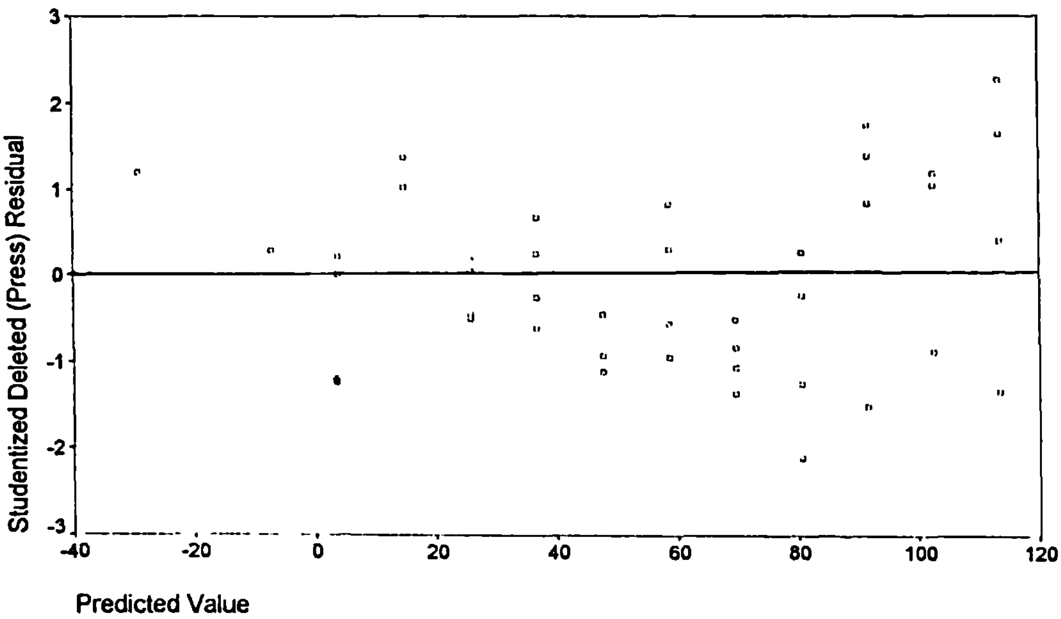
Figure 5.152: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Total Non-Haversian Canal Count (√ALLN-HAV) And Cement Layer Age



Regression of average non-Haversian canal count on cement layer age (n=42):

- The studentised deleted residuals were distributed around 0 (Figure 5.153), but their distribution was not random, and their variability increased with an increase in predicted value. This indicated that they did not have constant variance and, that, it was not appropriate to fit a linear regression model. Instead, the count was transformed and a new model was fitted to the relationship between average non-Haversian canal count and cement layer age (below).

Figure 5.153: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count



Regression of transformed average non-Haversian canal count on cement layer age (n=42):

- The K-S (Lilliefors) significance was >0.20 (Table 5.43) and the majority of points in the Q-Q plot (Figure 5.154) fell along a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.155). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 2.42 (Table 5.43). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was -0.75 (P=0.00) (Table 5.43). This indicated that there was a highly significant, strong degree of linear association between the two variables.
- The value of r^2 was 0.56 (Table 5.43). This indicated that the model fitted the data well and, that, 56% of a change in transformed average non-Haversian canal count could be accounted for by a change in cement layer age.

Table 5.43: Regression Of Transformed Average Non-Haversian Canal Count On Cement Layer

Age (n=42)

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
-0.74	11.34	− 0.75 (P=0.00)	0.56	>0.20	2.42

Figure 5.154: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

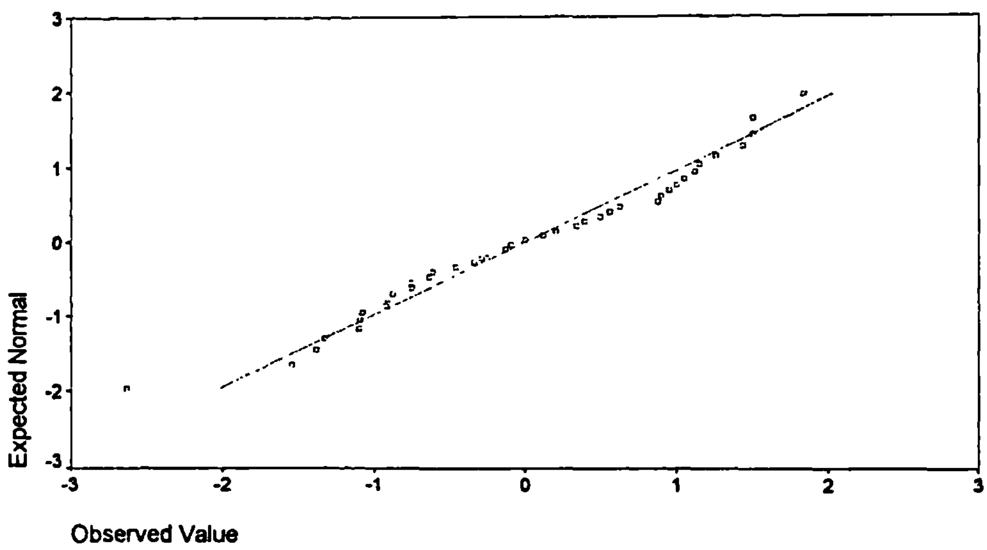


Figure 5.155: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

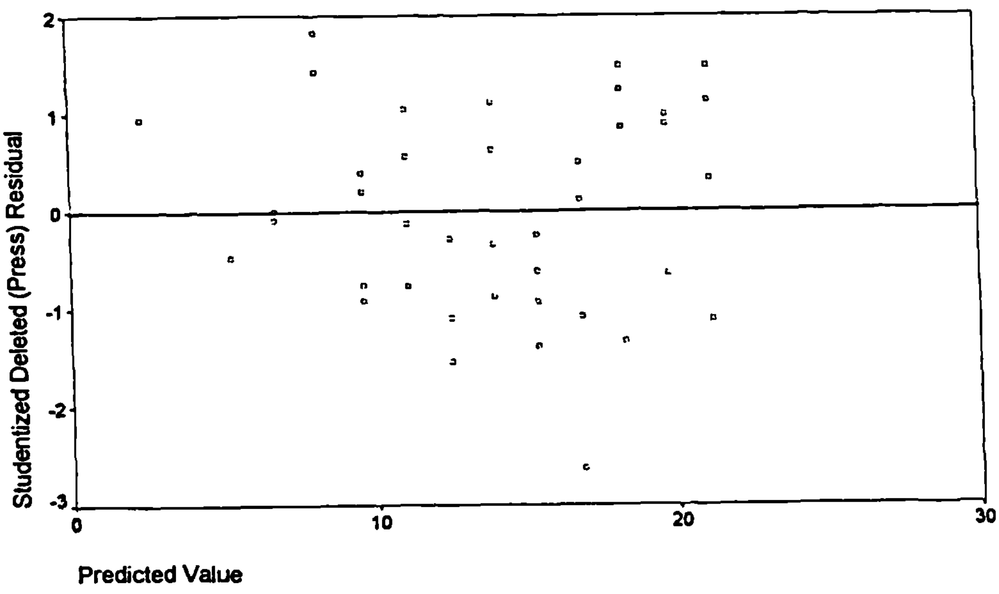
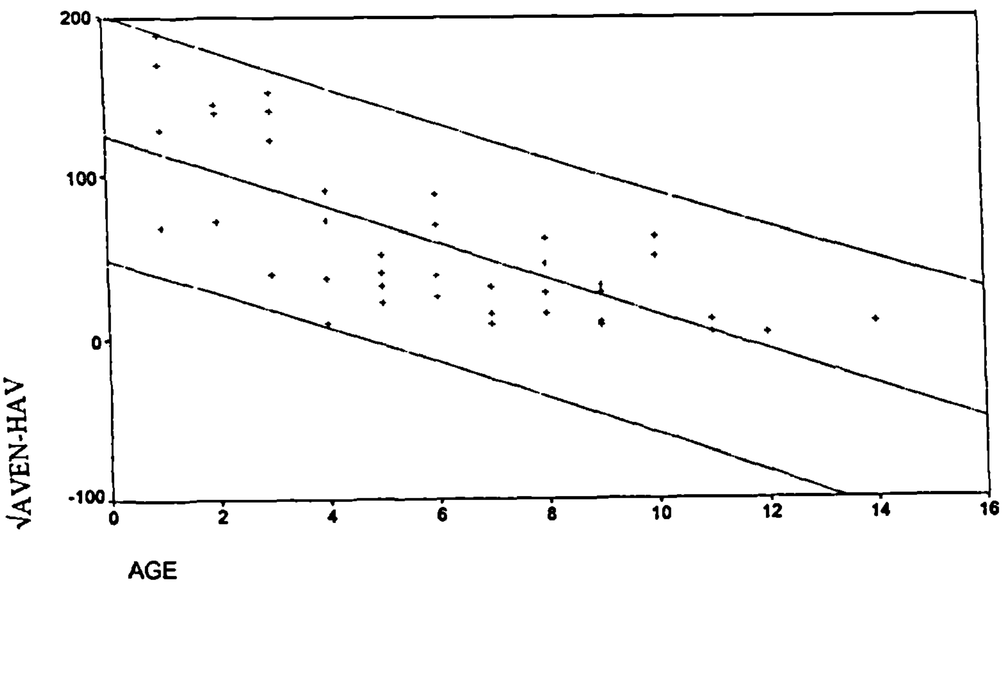


Figure 5.156: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Transformed Average Non-Haversian Canal Count (√AVEN-HAV) And Cement Layer Age



5.3.4 IN SUMMARY

Linear regression of secondary osteon counts on cement layer age for the whole sample (n=72):

- The studentised residuals for three secondary osteon counts were not normally distributed (position 2, position 3 and the total count) and these counts had to be transformed, using a square root transformation, before linear regression models could be fitted.
- After appropriate transformations, regression analysis revealed that all counts had positive, highly significant, weak linear associations with cement layer ages. Pearson's correlation coefficients ranged from 0.47 (P=0.00), for the count in position 4, to 0.57 (P=0.00) for the transformed count in position 3.
- The regression models did not fit the relationships between secondary osteon counts and cement layer age well. The value of r^2 ranged from a low of 0.22, for the position 4 count, to a high of 0.32, for the transformed position 3 count.

Linear regression of non-Haversian canal counts on cement layer age for the whole sample (n=72):

- The studentised deleted residuals for three non-Haversian canal counts did not have constant variance (position 1, position 2 and position 3) and, for the other three counts, they were not normally distributed (position 4 and the total and average counts). Consequently, all counts had to be transformed, using a square root transformation, before linear regression models could be fitted.
- After appropriate transformations, regression analysis revealed that all counts had negative, highly significant, weak to moderate degrees of linear association with cement layer ages. Pearson's correlation coefficients ranged from -0.53 (P=0.00), for the transformed position 4 count, to -0.68 (P=0.00), for the transformed total and average counts.
- On the whole, the regression models did not fit the relationships between transformed non-Haversian canal counts and cement layer age badly. For the transformed count in position 1, and the transformed total and average counts r^2 >0.40.

Linear regression of secondary osteon counts on cement layer age for the bucks (n=30):

- One count, that in position 3 did not have normally distributed studentised deleted residuals, and needed to be transformed, using a square root transformation, before a linear regression model could be fitted.
- After the appropriate transformation, regression analysis revealed that all counts had positive but very low, and in one case (position 4) an insignificant, degree of linear association with cement layer age. Pearson's correlation coefficients ranged from 0.15 (P=0.44), for the position 4 count, to 0.41(P=0.03), for the position 1 count.
- The regression models did not fit the relationships between secondary osteon counts and cement layer age well. The value of r^2 ranged from 0.02, for the position 4 count, to 0.16, for the position 1 count.

Linear regression of non-Haversian canal counts on cement layer age for the bucks (n=30):

- All counts met the assumptions of linear regression, as assessed through analysis of their studentised deleted residuals, and none required a transformation.
- Regression analysis revealed that all counts had negative but low, and in one case (position 4) an insignificant, degree of linear association with cement layer age. Pearson's correlation coefficients ranged from -0.21 (P=0.28), for the position 4 count, to -0.44 (P=0.02), for the position 3 count and the total and average counts.
- The regression models did not fit the relationships between non-Haversian canal counts and cement layer age well. The value of r^2 ranged from 0.05, for the position 4 count, to 0.19, for the position 3 count and the total and average counts.

Linear regression of secondary osteon counts on cement layer age for the does (n=42):

- All counts met the assumptions of linear regression, as assessed through analysis of their studentised deleted residuals, and none required a transformation.

- Regression analysis revealed that all counts had positive, highly significant, weak to moderate degrees of linear association with cement layer age. Pearson's correlation coefficients ranged from 0.47 (P=0.00), for the position 1 count, to 0.62 (P=0.00), for the position 3 count.
- The regression models did not fit the relationships between secondary osteon counts and cement layer age very well. The value of r^2 ranged from 0.22, for the position 1 count, to 0.38, for the position 3 count.

Linear regression of non-Haversian canal counts on cement layer age for the does (n=42):

- Not all counts had constant variance and needed to be transformed, using a square root transformation, before a linear regression model could be fitted.
- After appropriate transformations, regression analysis revealed that all counts, but one (position 2) had negative, highly significant, moderate to strong degrees of linear association with cement layer age. Pearson's correlation coefficients ranged from -0.59 (P=0.00), for the position 2 count, to -0.75 (P=0.00), for the total and average counts.
- The regression models fitted the relationships between transformed non-Haversian canal counts and cement layer age quite well. The value of r^2 ranged from 0.35, for the position 2 count, to 0.56 for the total and average counts.

5.3.5 Discussion

All counts (some only when transformed) showed varying degrees of linear association with cement layer age. Although a non-parametric test of association, Spearman's rank correlation had shown that the strongest degree of association between cement layer age and histological count was for non-Haversian canal counts in does. This trend was repeated here, where an attempt was made to fit a model to the relationships. Again, and as in Kerley (1965), secondary osteons were found to increase in number as cement layer age increased, and non-Haversian canals were found to decrease in number as cement layer age increased. The strongest degree of association between variables was Pearson's r = -0.75 (P=0.00) for transformed total and average non-Haversian canal counts with cement layer age in the does, and this compared well with the correlation coefficient of -0.79

(significance not stated) reported by Kerley (1965) through the regression of age on non-Haversian canal count in the tibia in humans (sexes combined), although it is noted that Kerley (1965) had a larger sample size (n=117) than that used here (n=72). Generally, the regression models did not fit the data well and the best fit was for the total and average non-haversian canal counts, where 56% of a change in their values could be accounted for by a change in cement layer age.

5.3.6 In Conclusion

- Non-Haversian canals showed stronger linear relationships than secondary osteons with cement layer age.
- Doe non-Haversian canal counts showed *stronger linear relationships* with cement layer age than buck non-Haversian canal counts.
- The 'best' fit of regression models were for the regressions of doe total and average non-Haversian canal counts on cement layer age, where 56% of a change in each count could be accounted for by a change in cement layer age.

5.4 New Cement Layer Ages Inferred From Bone Histology

Regression equations generated through the application of the above models were reversed to allow new cement layer ages for each specimen to be predicted from each histological count. For example, the regression equation derived from the fit of the linear regression model to the relationship between secondary osteon count in position 1, for the whole sample, (y) and cement layer age (x) was:

$y=14.08+3.40x$

Turning it round, so that secondary osteon count in position 1, for the whole sample, could be used to predict cement layer age, it became:

$(y-14.08)/3.4=x$

The mean inaccuracy and mean bias (in years) of resulting estimated cement ages for each year of life, and the total inaccuracy and total bias (in years) of each count for estimating all cement layer age were calculated (Tables 5.44 to 5.79). Inaccuracy is "the average absolute error of age estimation ..., without reference to over- or underageing" (Lovejoy *et al.*, 1985, p. 7). For example, an inaccuracy of 4.77 years, as shown for secondary osteon counts in position 1 for ageing all animals in the whole sample, (Table 5.44) is the simple mean absolute difference between actual cement layer age and predicted cement layer age as inferred from secondary osteon count in position 1. Bias is "the mean over or underprediction" (Lovejoy *et al.*, 1985, p. 7) and, therefore, can be positive or negative. For example, a bias of -3.30 years, as shown for secondary osteon counts in position 1 for ageing animals in their first year of life in the whole sample (Table 5.44), indicates that, for this group of animals, cement layer ages inferred from secondary osteon counts in position 1 are on average -3.30 years less than actual cement layer ages. Lovejoy *et al.* (1985) viewed inaccuracy and bias as more effective indicators of the reliability and accuracy of ageing methods than the correlation coefficient, which is dependent on the age range and composition of the test sample. Bias also allows trends, such as constant under or overprediction of age to be identified.

The Whole Sample (n=72)

Table 5.44: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST1*-14.08)/3.40=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	4.81	-3.30
2 (n=8)	2.70	1.21
3 (n=8)	5.08	-2.40
4 (n=8)	5.10	3.55
5 (n=8)	5.77	1.93
6 (n=8)	5.59	0.59
7 (n=3)	11.09	5.23
8 (n=7)	2.85	-0.59
9 (n=5)	5.16	-2.38
10 (n=5)	4.07	-3.38
11 (n=2)	2.35	1.62
12 (n=1)	2.09	2.09
14 (n=1)	2.55	-2.55
All (n=72)	4.77	-0.04

Table 5.45: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√OST2*-2.71)/0.49=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	6.32	-3.25
2 (n=8)	4.84	1.36
3 (n=8)	4.43	-0.80
4 (n=8)	4.95	4.67
5 (n=8)	5.60	1.65
6 (n=8)	5.17	-2.52
7 (n=3)	4.60	-1.03
8 (n=7)	2.67	-1.12
9 (n=5)	3.31	1.66
10 (n=5)	2.24	-2.24
11 (n=2)	2.47	2.47
12 (n=1)	0.26	0.26
14 (n=1)	2.83	-2.83
All (n=72)	4.45	-0.02

Table 5.46: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√OST3*-1.44)/0.53=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	3.55	-2.48
2 (n=8)	4.57	2.36
3 (n=8)	3.78	-1.36
4 (n=8)	4.29	2.27
5 (n=8)	5.25	-0.31
6 (n=8)	3.25	-0.63
7 (n=3)	4.39	4.39
8 (n=7)	3.32	-1.06
9 (n=5)	1.74	0.69
10 (n=5)	2.82	-1.23
11 (n=2)	1.61	1.61
12 (n=1)	1.07	1.07
14 (n=1)	3.38	-3.38
All (n=72)	3.67	0.04

Table 5.47: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: OST4*-16.06)/3.38=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	8.00	-4.19
2 (n=8)	4.87	1.16
3 (n=8)	5.18	1.76
4 (n=8)	4.27	2.93
5 (n=8)	4.53	-0.57
6 (n=8)	4.86	-0.63
7 (n=3)	2.57	2.57
8 (n=7)	4.93	-0.20
9 (n=5)	2.15	0.27
10 (n=5)	1.76	-1.76
11 (n=2)	1.98	1.98
12 (n=1)	5.49	-5.49
14 (n=1)	2.32	-2.32
All (n=72)	4.54	-0.05

Table 5.48: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√ALLOST*-5.72)/0.87=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	5.74	-3.65
2 (n=8)	4.33	2.00
3 (n=8)	3.63	-0.46
4 (n=8)	4.19	3.37
5 (n=8)	4.80	0.73
6 (n=8)	3.53	-0.77
7 (n=3)	3.82	2.32
8 (n=7)	2.49	-0.63
9 (n=5)	2.41	0.25
10 (n=5)	2.48	-2.48
11 (n=2)	1.63	1.63
12 (n=1)	0.44	-0.44
14 (n=1)	3.10	-3.10
All (n=72)	3.76	-0.02

Table 5.49: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (AVEOST*-12.25)/3.91=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	3.89	-2.22
2 (n=8)	3.74	1.35
3 (n=8)	4.25	-1.16
4 (n=8)	4.52	3.01
5 (n=8)	5.64	0.47
6 (n=8)	3.47	-0.19
7 (n=3)	5.30	2.68
8 (n=7)	3.19	-1.22
9 (n=5)	3.55	0.31
10 (n=5)	3.29	-3.29
11 (n=2)	3.77	3.77
12 (n=1)	0.79	0.79
14 (n=1)	2.36	-2.36
All (n=72)	4.00	0.00

Table 5.50: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV1*-10.31)/-0.70=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	3.13	-1.41
2 (n=8)	2.90	-0.20
3 (n=8)	5.10	-0.25
4 (n=8)	2.92	2.31
5 (n=8)	2.28	1.26
6 (n=8)	3.04	-1.29
7 (n=3)	5.59	4.72
8 (n=7)	2.59	-1.74
9 (n=5)	3.56	0.04
10 (n=5)	2.13	-0.23
11 (n=2)	0.55	0.55
12 (n=1)	2.73	2.73
14 (n=1)	2.77	-2.77
All (n=72)	3.13	0.08

Table 5.51: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV2-11.74)/-0.74=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	3.25	-0.71
2 (n=8)	4.09	-0.68
3 (n=8)	4.25	-0.73
4 (n=8)	3.25	1.88
5 (n=8)	5.49	1.24
6 (n=8)	4.94	-0.20
7 (n=3)	2.17	2.17
8 (n=7)	4.75	-1.90
9 (n=5)	3.14	1.44
10 (n=5)	2.74	-1.72
11 (n=2)	0.49	0.49
12 (n=1)	2.51	2.51
14 (n=1)	2.19	-2.19
All (n=72)	3.86	0.03

Table 5.52: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV3*-13.57)/-0.75=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	1.36	-0.44
2 (n=8)	3.82	0.98
3 (n=8)	2.16	-0.96
4 (n=8)	3.71	0.49
5 (n=8)	3.59	0.29
6 (n=8)	3.55	-1.61
7 (n=3)	6.11	6.11
8 (n=7)	3.31	-1.05
9 (n=5)	1.53	0.44
10 (n=5)	3.81	-1.51
11 (n=2)	1.56	1.56
12 (n=1)	3.11	3.11
14 (n=1)	1.72	-1.72
All (n=72)	3.08	0.00

Table 5.53: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV4*-9.72)/-0.58=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	4.15	-3.32
2 (n=8)	2.46	1.84
3 (n=8)	5.13	1.05
4 (n=8)	4.27	3.84
5 (n=8)	4.08	-1.61
6 (n=8)	4.78	-1.20
7 (n=3)	2.16	2.16
8 (n=7)	5.35	-2.34
9 (n=5)	3.90	1.58
10 (n=5)	2.12	-0.61
11 (n=2)	0.71	0.49
12 (n=1)	0.90	0.90
14 (n=1)	0.69	-0.69
All (n=72)	3.81	-0.03

Table 5.54: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√ALLN-HAV*-23.18)/-1.37=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	2.20	-1.23
2 (n=8)	2.57	0.62
3 (n=8)	3.51	-0.38
4 (n=8)	3.04	1.93
5 (n=8)	3.18	0.23
6 (n=8)	3.08	-1.15
7 (n=3)	3.86	3.86
8 (n=7)	3.49	-1.58
9 (n=5)	2.16	0.78
10 (n=5)	2.18	-1.81
11 (n=2)	1.16	1.16
12 (n=1)	2.50	2.50
14 (n=1)	1.58	-1.58
All (n=72)	2.85	-0.01

Table 5.55: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√AVEN-HAV*-11.62)/-0.69=x

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	3.25	-2.05
2 (n=8)	3.10	0.76
3 (n=8)	4.19	-0.49
4 (n=8)	3.32	2.41
5 (n=8)	3.61	0.66
6 (n=8)	3.24	-0.76
7 (n=3)	3.54	3.54
8 (n=7)	3.28	-1.34
9 (n=5)	1.58	0.73
10 (n=5)	1.70	-1.33
11 (n=2)	0.56	0.56
12 (n=1)	0.03	0.03
14 (n=1)	2.61	-2.61
All (n=72)	3.06	0.00

The Bucks (n=30)

Table 5.56: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST1*-13.32)/2.98=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	4.71	-4.71
2 (n=4)	4.44	4.44
3 (n=4)	6.12	0.67
4 (n=4)	5.20	2.94
5 (n=4)	4.90	-2.42
6 (n=4)	7.58	-0.07
8 (n=3)	4.12	-1.06
9 (n=1)	5.42	-5.42
10 (n=2)	2.64	2.64
All (n=30)	5.16	0.00

Table 5.57: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: OST2*-17.17)/2.29=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	18.71	-18.71
2 (n=4)	13.00	12.54
3 (n=4)	26.50	11.54
4 (n=4)	25.02	21.79
5 (n=4)	13.48	-11.71
6 (n=4)	20.50	-4.96
8 (n=3)	23.49	9.54
9 (n=1)	2.46	-2.46
10 (n=2)	20.04	20.04
All (n=30)	19.39	3.61

Table 5.58: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√OST3*-1.70)/0.30=x

AgeGroup	Mean Inacc.	Mean Bias
1 (n=4)	5.83	-5.83
2 (n=4)	10.82	10.82
3 (n=4)	8.74	0.07
4 (n=4)	7.33	2.50
5 (n=4)	6.87	-6.87
6 (n=4)	6.98	-3.02
8 (n=3)	6.48	-3.42
9 (n=1)	2.65	2.65
10 (n=2)	7.89	7.89
All (n=30)	7.47	-0.04

Table 5.59: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST4*-21.35)/1.22=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	18.27	-8.25
2 (n=4)	13.44	6.32
3 (n=4)	21.92	13.31
4 (n=4)	11.14	3.91
5 (n=4)	13.69	13.69
6 (n=4)	11.39	-5.19
8 (n=3)	17.52	-1.18
9 (n=1)	4.37	-4.37
10 (n=2)	10.61	10.61
All (n=30)	14.82	0.01

Table 5.60: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (ALLOST*-58.61)/8.35=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	6.01	-6.01
2 (n=4)	7.30	7.30
3 (n=4)	9.49	3.54
4 (n=4)	7.34	3.59
5 (n=4)	6.48	-6.48
6 (n=4)	5.66	-3.40
8 (n=3)	8.20	-1.21
9 (n=1)	3.56	-3.56
10 (n=2)	6.57	6.57
All (n=30)	7.05	0.00

Table 5.61: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (AVEOST*-14.75)/2.26=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.67	-5.67
2 (n=4)	6.61	6.55
3 (n=4)	8.77	3.00
4 (n=4)	6.67	2.97
5 (n=4)	6.41	-6.41
6 (n=4)	8.40	0.05
8 (n=3)	7.79	-1.77
9 (n=1)	4.02	-4.02
10 (n=2)	5.27	5.27
All (n=30)	6.89	0.00

Table 5.62: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (N-HAV1*-90.99)/-6.43=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.19	-3.25
2 (n=4)	6.77	0.96
3 (n=4)	6.86	1.57
4 (n=4)	4.31	3.73
5 (n=4)	2.56	1.19
6 (n=4)	6.62	-2.93
8 (n=3)	4.98	-2.32
9 (n=1)	2.69	-2.69
10 (n=2)	3.25	3.25
All (n=30)	5.11	0.06

Table 5.63: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (N-HAV2*-141.24)/-9.85=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	3.58	-3.16
2 (n=4)	4.62	2.09
3 (n=4)	6.46	1.92
4 (n=4)	4.79	2.80
5 (n=4)	4.44	-3.68
6 (n=4)	5.53	1.23
8 (n=3)	7.16	-3.14
9 (n=1)	1.06	-1.06
10 (n=2)	2.87	2.87
All (n=30)	4.87	0.00

Table 5.64: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (N-HAV3*-173.19)/-9.18=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	2.51	-1.52
2 (n=4)	7.02	4.67
3 (n=4)	3.45	-0.91
4 (n=4)	3.81	-0.22
5 (n=4)	5.78	0.06
6 (n=4)	4.51	-3.64
8 (n=3)	4.40	-1.81
9 (n=1)	4.20	4.20
10 (n=2)	3.75	3.75
All (n=30)	4.44	0.00

Table 5.65: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (N-HAV4*-98.99)/-4.42=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	8.82	-7.51
2 (n=4)	5.63	5.63
3 (n=4)	14.11	4.58
4 (n=4)	10.70	10.70
5 (n=4)	9.57	-6.98
6 (n=4)	12.86	-4.27
8 (n=3)	15.26	-6.57
9 (n=1)	1.08	-1.08
10 (n=2)	9.23	9.23
All (n=30)	10.30	-0.01

Table 5.66: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (ALLN=HAV*-506.37)/-30.07=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	3.17	-1.08
2 (n=4)	4.22	-1.11
3 (n=4)	6.10	1.43
4 (n=4)	4.80	2.10
5 (n=4)	3.96	1.63
6 (n=4)	5.79	1.08
8 (n=3)	6.96	2.48
9 (n=1)	0.26	3.27
10 (n=2)	4.16	0.17
All (n=30)	4.68	-0.01

Table 5.67: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (AVEN-HAV*-126.60)/-7.52=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	3.16	-3.16
2 (n=4)	4.22	3.25
3 (n=4)	6.09	1.46
4 (n=4)	4.80	2.92
5 (n=4)	3.96	-1.88
6 (n=4)	5.79	-2.35
8 (n=3)	6.96	-2.98
9 (n=1)	0.26	0.26
10 (n=2)	4.16	4.16
All (n=30)	4.68	0.00

The Does (n=42)

Table 5.68: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST1*-16.38)/3.30=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.63	-2.86
2 (n=4)	2.42	-2.42
3 (n=4)	5.54	-5.54
4 (n=4)	5.52	4.82
5 (n=4)	6.48	6.48
6 (n=4)	3.91	1.76
7 (n=3)	11.26	4.91
8 (n=4)	2.65	0.14
9 (n=4)	5.08	-1.92
10 (n=3)	6.78	-6.78
11 (n=2)	2.42	1.31
12 (n=1)	1.82	1.82
14 (n=1)	2.90	-2.90
All (n=42)	5.13	0.01

Table 5.60: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST2*-17.26)/4.72=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	6.83	-0.15
2 (n=4)	4.92	-2.34
3 (n=4)	4.91	-4.91
4 (n=4)	5.07	4.42
5 (n=4)	7.50	7.50
6 (n=4)	4.24	-1.92
7 (n=3)	4.00	-2.54
8 (n=4)	2.81	-2.81
9 (n=4)	4.32	3.39
10 (n=3)	6.03	-6.03
11 (n=2)	3.78	3.78
12 (n=1)	0.44	0.44
14 (n=1)	3.46	-3.46
All (n=42)	4.82	0.00

Table 5.70: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST3*-6.67)/4.74=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	2.15	-1.46
2 (n=4)	4.92	-2.34
3 (n=4)	4.91	-4.91
4 (n=4)	5.85	3.19
5 (n=4)	4.07	3.40
6 (n=4)	3.06	0.50
7 (n=3)	4.30	3.76
8 (n=4)	2.58	0.14
9 (n=4)	1.79	-0.18
10 (n=3)	6.19	-4.37
11 (n=2)	1.52	1.52
12 (n=1)	1.36	1.36
14 (n=1)	4.86	-4.86
All (n=42)	3.40	0.00

Table 5.71: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (OST4*-15.66)/3.95=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.01	-2.43
2 (n=4)	4.18	-1.41
3 (n=4)	3.10	-1.90
4 (n=4)	4.75	2.79
5 (n=4)	2.80	2.62
6 (n=4)	4.26	1.05
7 (n=3)	1.41	1.36
8 (n=4)	4.90	1.83
9 (n=4)	2.56	0.58
10 (n=3)	4.09	-4.09
11 (n=2)	3.29	2.76
12 (n=1)	7.61	-7.61
14 (n=1)	2.52	-2.52
All (n=42)	3.80	-0.01

Table 5.72: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (ALLOST*-56.14)/16.80=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	4.82	-1.60
2 (n=4)	3.71	-2.07
3 (n=4)	3.83	-3.83
4 (n=4)	4.90	3.71
5 (n=4)	4.92	4.92
6 (n=4)	2.11	0.15
7 (n=3)	4.63	1.58
8 (n=4)	1.41	-0.34
9 (n=4)	2.33	0.59
10 (n=3)	6.73	-6.73
11 (n=2)	2.33	2.33
12 (n=1)	0.52	-0.52
14 (n=1)	3.59	-3.59
All (n=42)	3.61	0.00

Table 5.73: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (AVEOST*-13.77)/4.23=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	4.58	-1.71
2 (n=4)	3.66	-2.00
3 (n=4)	3.76	-3.76
4 (n=4)	4.88	3.74
5 (n=4)	4.91	4.91
6 (n=4)	2.11	0.17
7 (n=3)	4.60	1.58
8 (n=4)	1.40	-0.34
9 (n=4)	2.31	0.58
10 (n=3)	6.70	-6.70
11 (n=2)	2.29	2.29
12 (n=1)	0.54	-0.54
14 (n=1)	3.60	-3.60
All (n=42)	3.57	0.00

Table 5.74: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV1*-10.63)/-0.79=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.27	-2.56
2 (n=4)	2.33	-1.47
3 (n=4)	5.91	-2.60
4 (n=4)	3.33	3.33
5 (n=4)	3.35	3.35
6 (n=4)	1.56	0.70
7 (n=3)	2.45	2.31
8 (n=4)	0.87	-0.01
9 (n=4)	1.37	0.05
10 (n=3)	1.51	-1.51
11 (n=2)	0.91	-0.91
12 (n=1)	1.44	-1.44
14 (n=1)	4.00	-4.00
All (n=42)	2.75	-0.01

Table 5.75: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV2*-11.10)/-0.73=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.00	-0.36
2 (n=4)	7.50	-4.43
3 (n=4)	5.21	-3.15
4 (n=4)	3.63	3.22
5 (n=4)	5.21	5.21
6 (n=4)	4.59	-0.62
7 (n=3)	2.16	2.16
8 (n=4)	2.37	-0.65
9 (n=4)	1.28	1.19
10 (n=3)	3.61	-3.61
11 (n=2)	0.60	-0.60
12 (n=1)	0.78	-0.78
14 (n=1)	3.47	-3.47
All (n=42)	3.77	0.00

Table 5.76: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV3*-13.22)/-0.81=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	2.42	-1.08
2 (n=4)	4.61	-1.99
3 (n=4)	2.60	-1.00
4 (n=4)	4.75	1.40
5 (n=4)	2.28	1.54
6 (n=4)	2.67	0.72
7 (n=3)	3.99	3.99
8 (n=4)	1.94	0.54
9 (n=4)	1.19	0.11
10 (n=3)	4.20	-3.00
11 (n=2)	0.32	-0.17
12 (n=1)	0.27	-0.27
14 (n=1)	3.27	-3.27
All (n=42)	2.82	0.00

Table 5.77: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√N-HAV4*-9.31)/-0.60=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	5.00	-3.86
2 (n=4)	3.35	1.69
3 (n=4)	4.05	-0.47
4 (n=4)	2.76	2.12
5 (n=4)	1.94	1.53
6 (n=4)	2.97	0.09
7 (n=3)	1.58	1.17
8 (n=4)	3.04	-1.14
9 (n=4)	2.33	2.33
10 (n=3)	3.10	-2.57
11 (n=2)	0.68	-0.57
12 (n=1)	0.21	-0.21
14 (n=1)	1.82	-1.82
All (n=42)	2.84	0.04

Table 5.78: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√ALLN-HAV*-22.60)/-1.45=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	3.14	-1.73
2 (n=4)	2.61	-1.61
3 (n=4)	3.83	-2.07
4 (n=4)	3.09	1.77
5 (n=4)	1.97	1.97
6 (n=4)	2.22	-0.78
7 (n=3)	2.49	2.49
8 (n=4)	1.87	-0.94
9 (n=4)	1.69	0.39
10 (n=3)	5.09	-5.09
11 (n=2)	0.28	-0.28
12 (n=1)	0.93	0.93
14 (n=1)	3.04	-3.04
All (n=42)	2.53	-0.39

Table 5.79: Inaccuracy And Bias Of Cement Layer Ages (x) Generated Through The Reverse Regression: (√AVEN-HAV*-11.34)/-0.74=x

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	2.88	-1.30
2 (n=4)	2.43	-1.20
3 (n=4)	3.57	-1.67
4 (n=4)	2.97	2.13
5 (n=4)	2.25	2.25
6 (n=4)	2.18	-0.47
7 (n=3)	2.72	2.72
8 (n=4)	1.83	-0.66
9 (n=4)	1.66	0.62
10 (n=3)	4.77	-4.77
11 (n=2)	0.91	0.71
12 (n=1)	1.08	1.08
14 (n=1)	2.84	-2.84
All (n=42)	2.50	-0.04

5.4.1 The Whole Sample

Secondary Osteon Counts

- Mean inaccuracy of cement layer ages inferred from secondary osteon counts ranged from 0.26 years, for animals in their 12th year of life aged through the transformed position 2 count (n=1), to 11.09 years, for animals in their 7th year of life aged through the position 1 count (n=3).
- Mean bias of cement layer ages inferred from secondary osteon counts ranged from -5.49 years, for animals in their 12th year of life aged through the position 4 count, to 5.23 years, for animals in their 7th year of life aged through the position 1 count.
- Total inaccuracy, for all age groups, ranged from 3.67 years, for transformed position 3 inferred ages, to 4.77 years, for position 1 inferred ages.
- Total bias, for all age groups, ranged from -0.05 years, for position 4 inferred cement layer ages, to 0.04 years, for transformed position 3 inferred cement layer ages.
- There were no trends to the bias of cement layer ages inferred from secondary osteon counts. That is, no count resulted in the constant under or overprediction of cement layer ages. However, all counts did result in the average underprediction of cement layer age of animals in their 1st, 8th, 10th and 14th years of life and average overprediction of cement layer age of those in their 2nd, 4th and llth years.

Non-Haversian Canal Counts

- Mean inaccuracy of cement layer ages inferred from non-Haversian canal counts ranged from 0.03 years, for animals in their 12th year of life aged through the transformed average count, to 6.11 years, for animals in their 7th year of life aged through the transformed position 3 count.
- Mean bias of cement layer ages inferred from non-Haversian canal counts ranged from -3.32 years, for animals in their 1st year of life aged through the transformed position 4 count, to 6.11 years, for animals in their 7th year of life aged through the transformed position 3 count.
- Total inaccuracy, for all age groups, ranged from 2.85 years, for transformed total inferred ages, to 3.86 years, for transformed position 2 inferred ages.

- Total bias, for all age groups, ranged from -0.03, for transformed position 4 inferred cement layer ages, to 0.08, for transformed position 1 inferred cement layer ages.
- There were no trends to the average bias of cement layer ages inferred from non-Haversian canal counts. That is, no count resulted in constant over or underprediction of cement layer ages. However, all counts resulted in the average underprediction of cement layer age for animals in their 1st, 6th, 8th, 10th and 14th years of life, and average overprediction for those in their 4th, 7th, 9th, 11th and 12th years of life.

5.4.2 THE BUCKS

Secondary Osteon Counts

- Mean inaccuracy of cement layer ages inferred from secondary osteon counts ranged from -2.46 years, for animals in their 10th year of life aged through the position 1 count, to 25.02 years, for animals in their 4th year of life aged through the position 2 count.
- Mean bias of cement layer ages inferred from secondary osteon counts ranged from -18.71 years, for animals in their 1st year of life aged through the position 2 count, to 21.79 years, for animals in their 4th year of life, aged through the position 4 count.
- Total inaccuracy, for all age groups, ranged from 5.16 years, for position 1 inferred ages, to 19.39 years, for position 2 inferred cement layer ages.
- Total bias, for all age groups, ranged from -0.04 years, for transformed position 3 ages, to 3.61 years for position 2 inferred cement layer ages.
- The average bias of cement layer ages inferred from secondary osteon counts showed specific trends; for the 1st year of life all counts produced cement layer age estimates that were on average smaller than actual cement layer ages, for the next three years they produced estimates that were on average larger than actual cement layer ages, between the 5th and 9th years inferred cement layer ages, mainly, were on average smaller than actual cement layer ages and, finally, for the 10th year all estimates were on average larger than actual cement layer ages.

Non-Haversian Canal Counts

- Mean inaccuracy of cement layer ages inferred from non-Haversian canal counts ranged from 0.26 years, for animals in their 9th year of life aged through total and average counts, to 15.26 years, for animals in their 8th year of life aged through the position 4 count.
- Mean bias of cement layer ages inferred from non-Haversian canal counts ranged from -7.51 years, for animals in their 1st year of life aged through the position 4 count, to 10.70 years, for animals in their 4th year of life aged through the position 4 counts.
- Total inaccuracy, for all age groups, ranged from 4.44 years, for position 2 inferred cement layer ages, to 10.30 years, for position 4 inferred ages.
- Total bias, for all age groups, ranged from -0.01 years, for position 4 and total inferred cement layer ages, to 0.06 years, for position 1 inferred cement layer ages.
- There were no trends to the average bias of cement layer ages inferred from non-Haversian canal counts. That is, no count resulted in constant over or underprediction of cement layer ages. However, all counts resulted in the average underprediction of cement layer age for animals in their 1st year of life, and in average overprediction for those in their 2nd and 10th years.

5.4.3 The Does

Secondary Osteon Counts

- Mean inaccuracy of cement layer ages inferred from secondary osteon counts ranged from 0.44 years, for animals in their 12th year of life aged through position 2 counts, to 11.26 years, for animals in their 7th year of life aged through position 1 counts.
- Mean bias of cement layer ages inferred from secondary osteon counts ranged from -7.61 years, for animals in their 12th year of life aged through position 4 counts, to 7.50 years, for animals in their 7th year of life aged through position 2 counts.
- Total inaccuracy, for all age groups, ranged from 3.40 years, for position 3 inferred cement layer ages, to 5.13 years, for position 1 inferred cement layer ages.
- Total bias, for all age groups, ranged from -0.01 years, for position 4 inferred ages, to 0.01 years for position 1 inferred cement layer ages.

- All counts produced cement layer ages that were on average consistently lower than actual cement layer ages for animals in their 1st, 2nd, 3rd, 10th and 14th years of life, and cement layer ages that were on average consistently higher than actual cement layer ages for animals in their 4th, 5th and 11th years of life.

Non-Haversian Canal Counts

- Mean inaccuracy of cement layer ages inferred from non-Haversian canal counts ranged from 0.21 years, for animals in their 12th year of life aged through transformed position 4 counts, to 7.50 years, for animals in their 2nd year of life aged through transformed position 2 counts.
- Mean bias of cement layer ages inferred from non-Haversian canal counts ranged from -5.09 years, for animals in their 10th year of life aged through transformed total counts, to 5.21 years, for animals in their 5th year of life aged through transformed position 2 counts.
- Total inaccuracy, for all age groups, ranged from 2.50 years, for transformed average inferred cement layer ages, to 3.77 years, for transformed position 2 inferred cement layer ages.
- Total bias, for all age groups, ranged from -0.39 years, for transformed total inferred cement layer ages, to 0.04 years, for transformed position 4 cement layer ages.
- All counts produced cement layer ages that were on average lower than actual cement layer ages for animals in their 1st, 2nd and 3rd years of life. Similarly, most counts (not always the total and/or average counts) produced cement layer ages that were on average lower than actual cement layer ages for the older animals, that is those between their 10th and 14th years of life. For animals in their 4th and 5th years, counts resulted in the production of cement layer ages that were on average higher than actual cement layer ages.

5.4.4 In Summary

- Non-Haversian canal counts for the does produced, on average, the most accurate cement layer ages, and average inaccuracy ranged from 2.50 years, for the average count, to 3.77 years, for the position 2 count.

- Secondary osteon counts for the bucks produced, on average, the least accurate cement layer ages, and average inaccuracy ranged from 6.89 years, for the average count, to 19.39 years, for the position 2 count.
- Most counts resulted in the average underprediction of cement layer age for the younger and older animals, and for the overprediction of cement layer age for those between their 4th and 8th years of life.

5.4.5 Discussion

The ageing technique developed in the current project was found to be more accurate for ageing does than bucks, and those counts that had shown the strongest correlations with cement layer age did produce, on average, the most accurate age estimates. Again, it is suggested that the technique is more appropriate for ageing does than bucks because of possible disruptions to growth and, consequently bone turnover, experienced by bucks during the rut and the production of antlers.

5.4.6 In Conclusion

- The most accurate estimations of cement layer age were made when average non-Haversian canal counts were used to estimate the age of does. However, this count resulted in the average underprediction of cement layer age for animals in 1st, 2nd, 3rd, 6th, 8th, 10th and 14th years, and in overprediction for animals in their 4th, 5th, 7th, 9th, 11th and 12th years.
- The least accurate age estimates of cement layer age were provided by secondary osteon counts for the bucks, for which all counts produced age estimates with an average inaccuracy >7.5 years.

5.5 Correlation Of Tooth Wear Scores With Cement Layer Ages

Tooth wear scores were correlated with cement layer ages for:

5.5.1 The Whole Sample

5.5.2 The Bucks

5.5.3 The Does

Non-parametric Spearman's rank correlation or linear Pearson's correlation was used as appropriate (Table 5.80).

Table 5.80: Correlation Of Tooth Wear Scores And Cement Layer Ages

	Correlation
Whole Sample (n=72)	0.88 (P=0.00) [S]
Bucks (n=30)	0.84 (P=0.00) [R]
Does (n=42)	0.84 (P=0.00) [R]

[R] - Pearson's correlation (linear)

[S] - Spearman's rank correlation (non-parametric)

5.5.1 The Whole Sample

- Tooth wear scores showed a positive, highly significant, strong degree of association with cement layer ages.
- The association between tooth wear scores and cement layer ages was non-parametric.
- The correlation coefficient was 0.88 (P=0.00).

5.5.2 The Bucks

- Tooth wear scores showed a positive, highly significant, strong degree of linear association with cement layer ages.
- The correlation coefficient was 0.84 (P=0.00).

5.5.3 The Does

- Tooth wear scores showed a positive, highly significant, strong degree of linear association with cement layer ages.
- The correlation coefficient was 0.84 (P=0.00).

5.5.4 In Summary

- Tooth wear scores were significantly and strongly associated with cement layer ages, for the whole sample and for the bucks and does separately.
- For the whole sample, there was a non-parametric strong association between tooth wear scores and cement layer ages.
- For the bucks and does, there were strong linear associations between tooth wear scores and cement layer ages.

5.5.5 Discussion

Tooth wear scores are known to increase with age (Chapter 1) and, here, they showed a strong degree of association with cement layer age. This increased the confidence in the cement layer ages provided for the roe deer by the Forestry Commission, and suggested that they were at least representative of a consistent measure of increase in true age. However, if this was true, tooth wear scores were generally better correlated with age than the secondary osteon and non-Haversian canal counts.

5.5.6 In Conclusion

Tooth wear scores showed a strong degree of association with cement layer age for the whole sample, and for the bucks and does.

5.6 Using Tooth Wear Scores To Predict Cement Layer Age

Tooth wear scores were regressed (linear regression) on cement layer age for:

5.6.1 The Whole Sample

5.6.2 The Bucks

5.6.3 The Does

Resulting regression equations were reversed and new cement layer ages were predicted for each specimen. The mean inaccuracy and mean bias (in years) of resulting estimated cement ages for each year of life, and the total inaccuracy and total bias (in years) of tooth wear scores for estimating all cement layer age were calculated.

5.6.1 The Whole Sample

Regression of tooth wear score on cement layer age (n=72):

- The K-S (Lilliefors) significance was close to 0.00. This indicated that the studentised deleted residuals were not normally distributed and, that, it was not appropriate to fit a linear regression model. After various transformations (square root, squaring, log and cubing the tooth wear scores), it was found that when a square root transformation was fitted to cement layer ages a linear regression model could be fitted to the relationship between tooth wear score and transformed cement layer age (below).

Regression of tooth wear score on transformed cement layer age (n=72):

- The K-S (Lilliefors) significance was >0.20 (Table 5.81) and the majority of plots in the Q-Q plot (Figure 5.157) fell on a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly distributed around 0 (Figure 5.158). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.56 (Table 5.81). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.88 (P=0.00) (Table 5.81). This indicated that there was a positive, highly significant, linear relationship between the two variables.
- The value of r^2 was 0.77 (Table 5.81). This indicated that the regression model fitted the relationship between the two variables well and, that, 77% of a change in tooth wear score could be accounted for by a change in transformed cement layer age.

Table 5.81: Regression Of Tooth Wear Score On Transformed Cement Layer Age (n=72);

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
22.50	7.05	0.88 (P=0.00)	0.77	>0.20	1.56

Figure 5.157: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

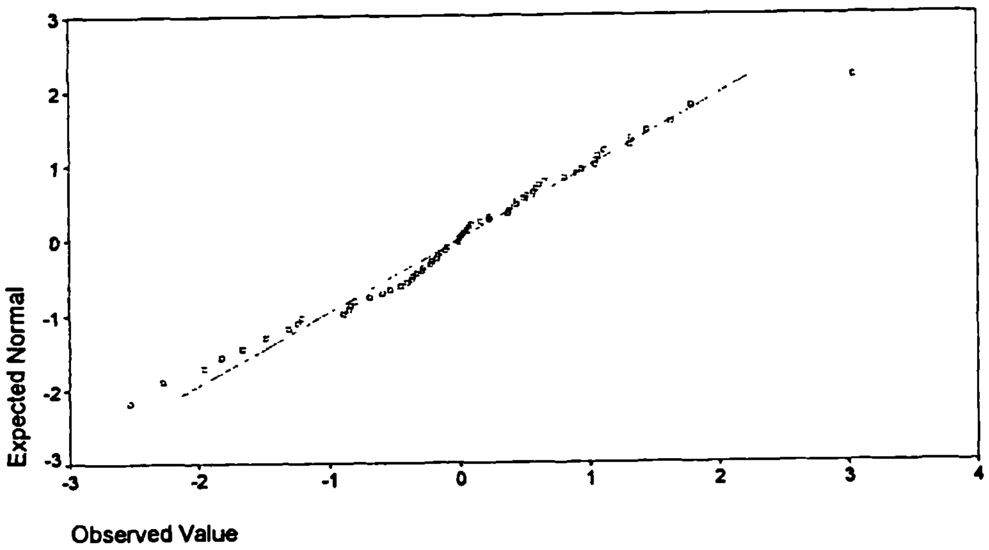


Figure 5.158: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

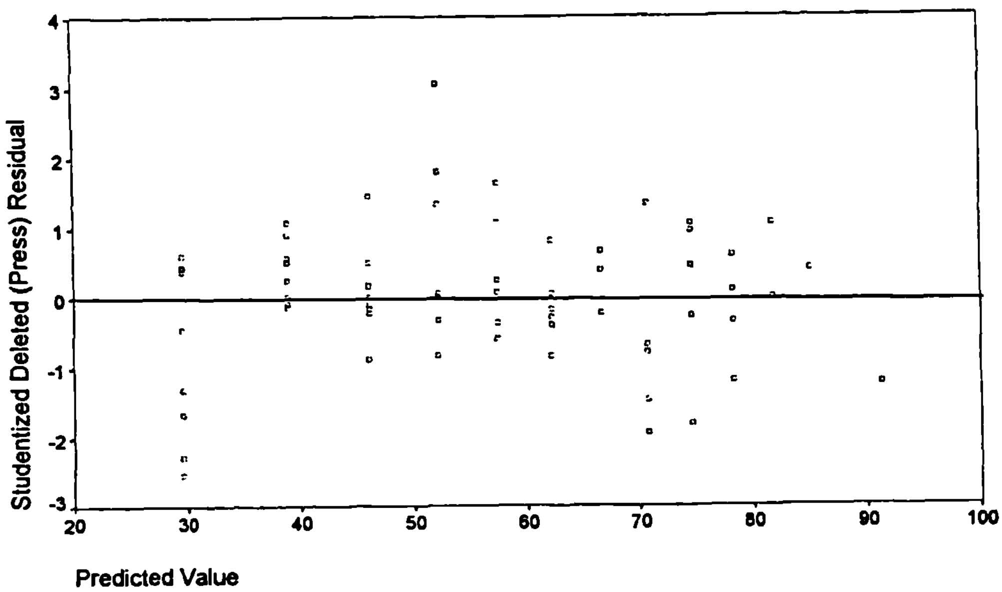
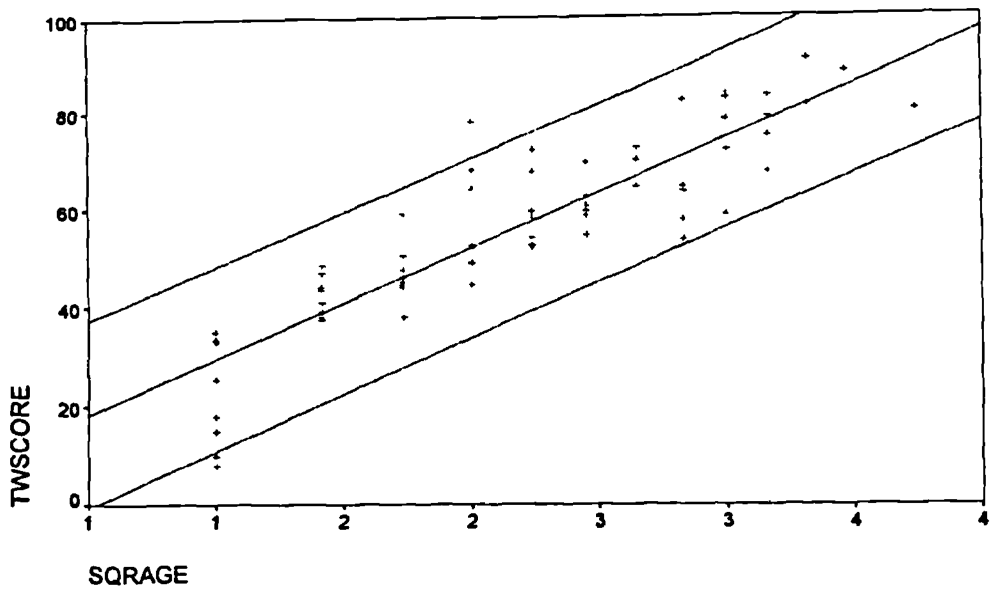


Figure 5.159: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Tooth Wear Score And √Cement Layer Age



The linear regression equation produced was y =7.05+22.50x, were y was tooth wear score and x was √cement layer age. To predict cement layer age this was reversed to $((y-7.05)/22.50)^2=x$, and the average inaccuracy and bias of new cement layer ages are presented in Table 5.82.

Table 5.82: Inaccuracy And Bias Of Cement Layer Ages Generated Through Reversed Regression Of Tooth Wear Score On Cement Layer Age

Age Group	Mean Inacc.	Mean Bias
1 (n=8)	0.65	-0.34
2 (n=8)	0.55	0.48
3 (n=8)	0.64	0.19
4 (n=8)	2.11	1.67
5 (n=8)	1.24	0.50
6 (n=8)	0.84	-0.25
7 (n=3)	0.92	0.60
8 (n=7)	2.69	-1.61
9 (n=5)	2.12	0.34
10 (n=5)	1.36	-0.48
11 (n=2)	1.49	1.43
12 (n=1)	1.10	1.10
14 (n=1)	3.35	-3.35
All (n=72)	1.31	0.16

- The mean inaccuracy of cement layer ages inferred from tooth wear scores ranged from 0.55 years, for animals in their 2nd year of life, to 3.55 years, for animals in their 14th year of life.
- The mean bias of cement layer ages inferred from tooth wear scores ranged from -3.35 years, for animals in their 14th year of life, to 1.67 years, for animals in their 4th year of life.
- Total inaccuracy, for all age groups, was 1.31 years.
- Total bias, for all age groups, was 0.16 years.
- There were no trends to the bias of cement layer ages inferred through tooth wear scores, that is they did not result in the constant under or overprediction of cement layer ages.

5.6.2 THE BUCKS

Regression of tooth wear scores on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.83) and the majority of points in the Q-Q plot (Figure 5.160) were distributed around a straight line. This indicated that the studentised deleted residuals were normally distributed.
- The studentised deleted residuals were randomly scattered around 0 (Figure 5.161). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.46 (Table 5.83). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.84 (P=0.00) (Table 5.83). This indicated that there was a positive, highly significant, linear relationship between the two variables.
- The value of r^2 was 0.71 (Table 5.83). This indicated that the regression model fitted the relationship between the two variables well and that 71% of a change in tooth wear score could be accounted for by a change in cement layer age.

Table 5.83: Regression Of Tooth Wear Score On Cement Layer Age (n=30);

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
3.93	33.54	0.84 (P=0.00)	0.71	>0.20	1.46

Figure 5.160: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

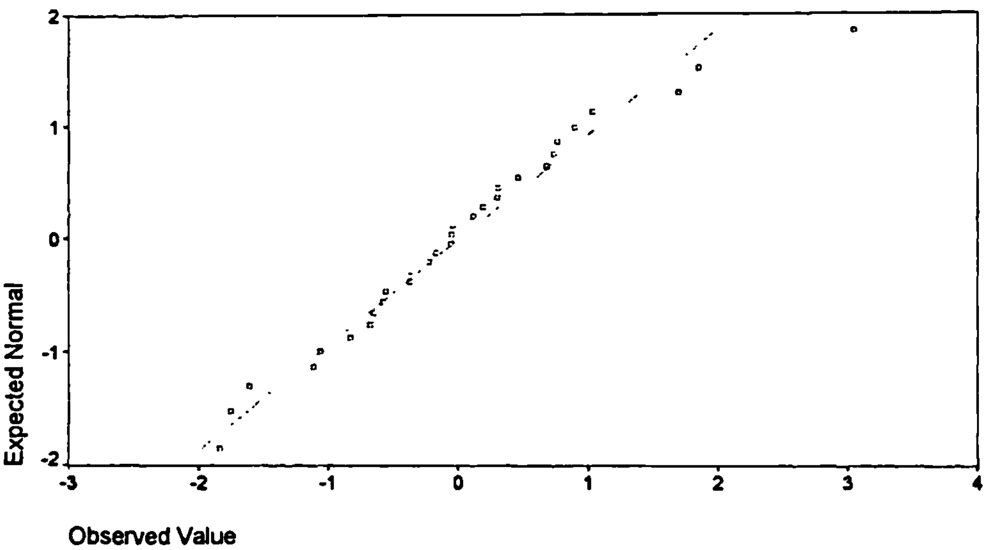


Figure 5.161: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

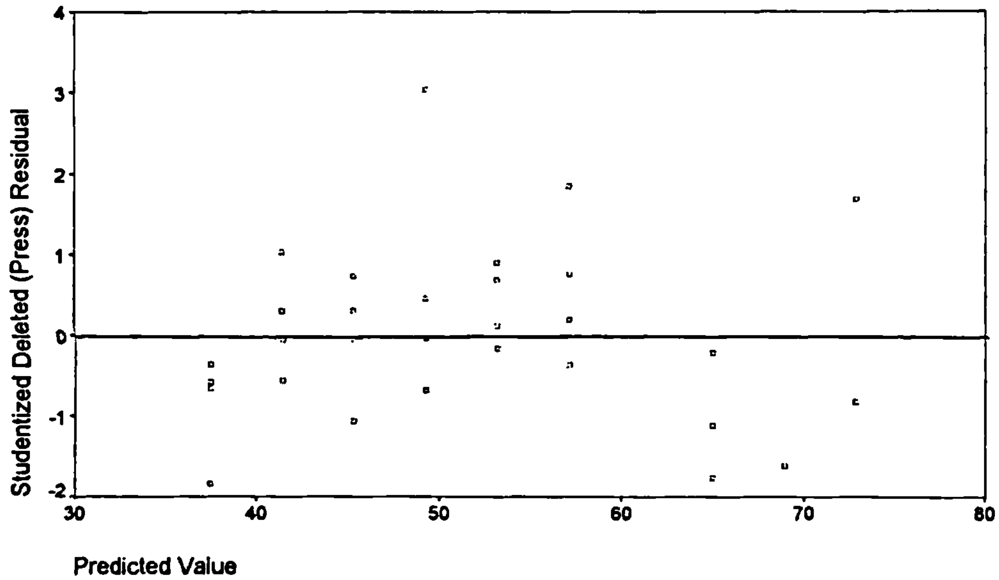
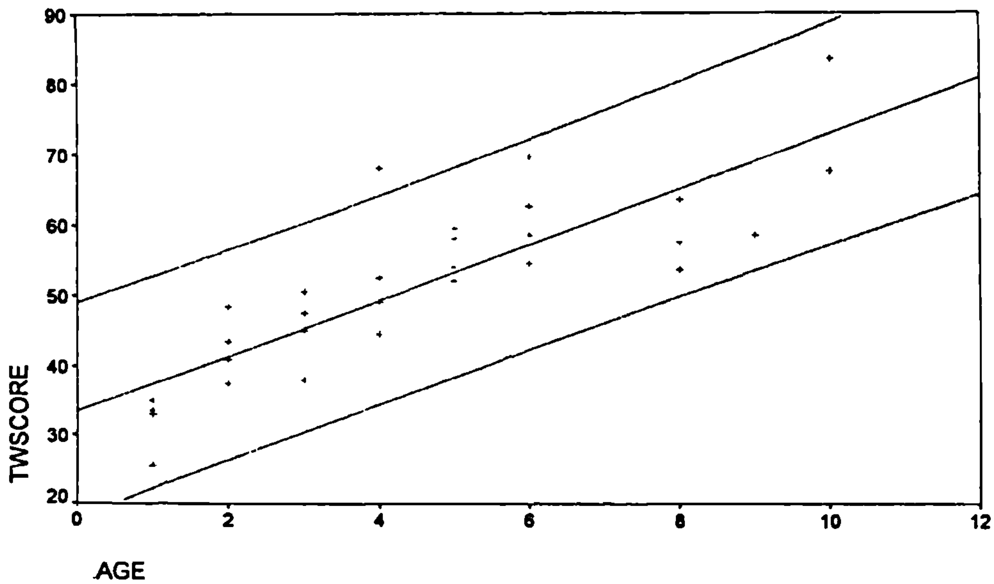


Figure 5.162: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Tooth Wear Score And Cement Layer Age



The linear regression equation produced was y =33.54+3.93x, were y was tooth wear score and x was cement layer age. To predict cement layer age this was reversed to (x-33.54)/3.93=x, and the average inaccuracy and bias of new cement layer ages are presented in Table 5.84.

Table 5.84: Inaccuracy And Bias Of Cement Layer Ages Generated Through Reversed Regression Of Tooth Wear Score On Cement Layer Age

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	1.45	-1.45
2 (n=4)	0.86	0.31
3 (n=4)	0.95	-0.02
4 (n=4)	1.72	1.07
5 (n=4)	0.83	0.68
6 (n=4)	1.38	1.04
8 (n=3)	1.74	-1.74
9 (n=1)	2.65	-2.65
10 (n=2)	2.03	0.67
All (n=30)	1.36	0.00

- The mean inaccuracy of cement layer ages inferred from tooth wear scores ranged from 0.83 years, for animals in their 5th year of life, to 2.65 years, for animals in their 9th year of life.
- The mean bias of cement layer ages inferred from tooth wear scores ranged from -2.65 years, for animals in their 9th year of life, to 1.07 years, for animals in their 4th year of life.
- Total inaccuracy, for all age groups, was 1.36 years.
- Total bias, for all age groups, was 0.00 years.
- There were no trends to the bias of cement layer ages inferred through tooth wear scores, that is they did not result in the constant under or overprediction of cement layer ages.

5.6.3 THE DOES

Regression of tooth wear scores on cement layer age (n=30):

- The K-S (Lilliefors) significance was >0.20 (Table 5.85). However, some points in the Q-Q plot (Figure 5.163) were not distributed on a straight line but, as the data adhered to all other assumptions necessary to fit a linear regression model (below), the studentised deleted residuals were accepted as normally distributed.

- The studentised deleted residuals were randomly scattered around 0 (Figure 5.164). This indicated that they had constant variance and a linear relationship with cement layer age.
- The Durbin-Watson value was 1.16 (Table 5.85). This indicated that the studentised deleted residuals were independent.
- The Pearson's correlation coefficient was 0.84 (P=0.00) (Table 5.85). This indicated that there was a positive, highly significant, strong degree of association between the two variables.
- The value of r^2 was 0.71 (Table 5.85). This indicated that the regression model fitted the relationship between the two variables well and, that, 71% of a change in tooth wear score could be accounted for by change in cement layer age.

Table 5.85: Regression Of Tooth Wear Score On Cement Layer Age (n=42);

Slope	Intercept	Pearson's Correlation Coefficient (r)	r^2	K-S Lilliefors Significance	Durbin-Watson
5.23	29.25	0.84 (P=0.00)	0.71	>0.20	1.16

Figure 5.163: Q-Q Plot Of Observed Against Expected Studentised Deleted Residuals

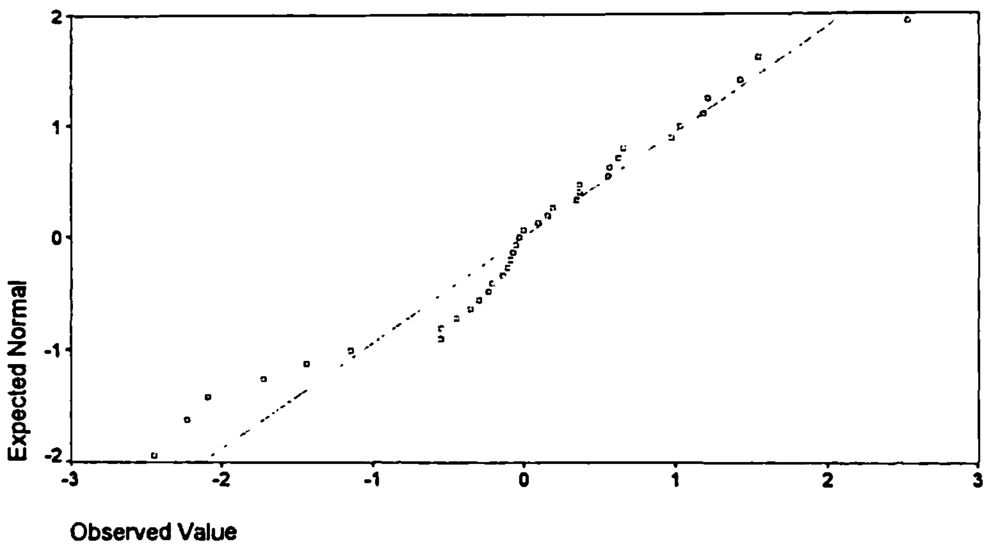


Figure 5.164: Scatterplot Of Studentised Deleted Residuals Against Predicted Value Of Count

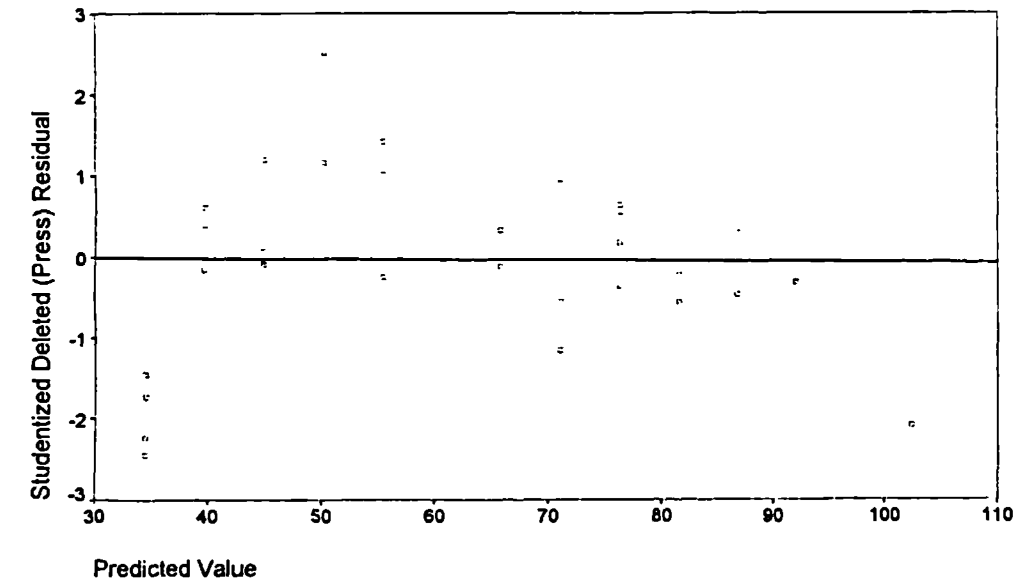
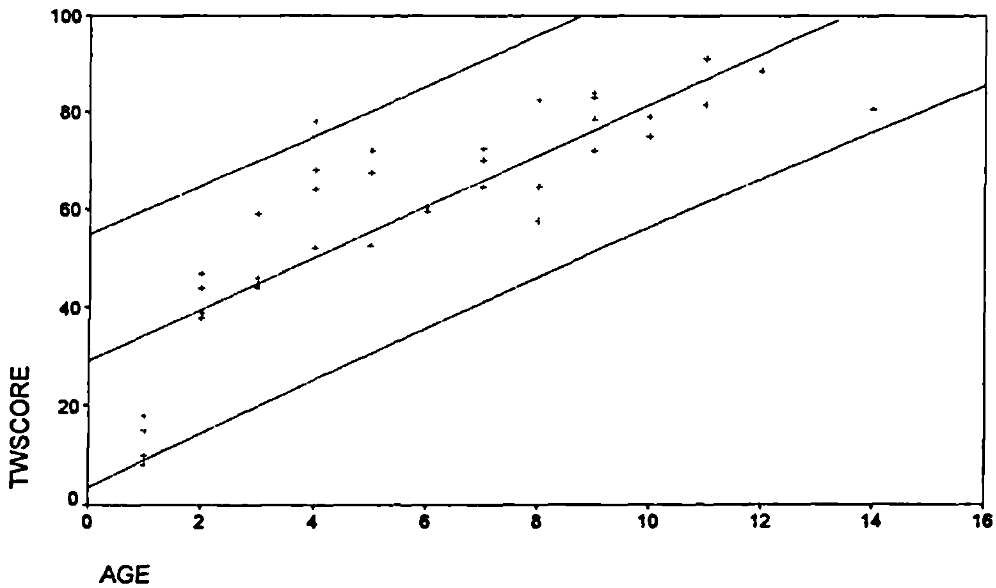


Figure 5.165: The Regression Line, With 95% Confidence Intervals, Fitted To The Relationship Between Tooth Wear Score And Cement Layer Age



The linear regression equation produced was y =29.25+5.23x, were y was tooth wear score and x was cement layer age. To predict cement layer age this was reversed to (y-29.25)/5.23=x, and the average inaccuracy and bias of new cement layer ages are presented in Table 5.86.

Table 5.86: Inaccuracy And Bias Of Cement Layer Ages Generated Through Reversed Regression Of Tooth Wear Score On Cement Layer Age

Age Group	Mean Inacc.	Mean Bias
1 (n=4)	4.16	-4.16
2 (n=4)	0.67	0.44
3 (n=4)	0.79	0.66
4 (n=4)	2.94	2.94
5 (n=4)	2.02	1.65
6 (n=4)	0.12	-0.12
7 (n=3)	0.78	0.61
8 (n=4)	2.01	-0.55
9 (n=4)	1.00	0.59
10 (n=3)	0.86	-0.86
11 (n=2)	0.91	-0.09
12 (n=1)	0.66	-0.66
14 (n=1)	4.19	-4.19
All (n=42)	1.66	0.00

- Mean inaccuracy of cement layer ages inferred from tooth wear scores range from 0.12 years, for animals in their 6th year of life, to 4.19 years, for animals in their 14th year of life.
- Mean bias of cement layer ages inferred from tooth wear scores ranged from -4.19 years, for animals in their 14th year of life, to 2.94 years, for animals in their 4th year of life.
- Total inaccuracy, for all age groups, was 1.66 years.
- Total bias, for all age groups, was 0.00 years.
- There were no trends to the bias of cement layer ages inferred through tooth wear scores, that is they did not result in the constant under or overprediction of cement layer ages.

5.6.4 In Summary

- Tooth wear scores showed positive, highly significant, strong degrees of linear association with cement layer age, for the whole sample, and the bucks and the does separately.
- The linear regression models fitted the relationships between tooth wear score and cement layer age very well, for the whole sample, the bucks and the does, and r^2 was >0.70.
- The most accurate estimates of cement layer age were provided by tooth wear scores for the whole sample.
- Cement layer ages inferred from tooth wear scores were not consistently higher or lower than actual cement layer ages.

5.6.5 Discussion

Tooth wear scores showed a positive, highly significant, strong degree of linear association with cement layer age (for the whole sample with transformed cement layer age). This was as expected, because tooth wear scores are known to increase with age and, again, this reinforces the credibility of the cement layer ages provided for the roe deer studied here, and the conclusions made about relationships between bone histology counts and cement layer ages.

5.6.6 In Conclusion

Tooth wear scores showed strong associations with cement layer ages for the whole sample, the bucks and the does, and for all three groups could be used to produce accurate new cement layer ages.

5.7 Correlation of Histological Feature Counts with Tooth Wear Scores

All secondary osteon and non-Haversian canal counts were further correlated with tooth wear scores, for:

5.7.1 The Whole Sample (Table 5.87)

5.7.2 The Bucks (Table 5.88)

5.7.3 The Does (Table 5.89)

Table 5.87: Correlation Of Secondary Osteon And Non-Haversian Canal Counts With Tooth Wear Scores, For The Whole Sample (n=72)

Feature Count*	CC	*P*	Feature Count*	CC	*P*
OST1	0.57^{R}	0.00	N-HAV1	-0.71^{R}	0.00
OST2	0.54^{R}	0.00	N-HAV2	-0.66^{R}	0.00
OST3	0.62^{R}	0.00	N-HAV3	-0.69^{R}	0.00
OST4	0.54^{R}	0.00	N-HAV4	-0.68^{S}	0.00
ALLOST	0.65^{R}	0.00	ALLN-HAV	-0.76^{R}	0.00
AVEOST	0.65^{R}	0.00	AVEN-HAV	-0.76^{R}	0.00

Table 5.88: Correlation Of Secondary Osteon And Non-Haversian Canal Counts With Tooth Wear Scores, For The Bucks (n=30)

Feature Count*	CC	*P*	Feature Count*	CC	*P*
OST1	0.50^{R}	0.00	N-HAV1	-0.50^{R}	0.00
OST2	0.41^{R}	0.02	N-HAV2	-0.59^{R}	0.00
OST3	0.40^{S}	0.03	N-HAV3	-0.48^{R}	0.00
OST4	0.25^{R}	0.20	N-HAV4	-0.34^{S}	0.07
ALLOST	0.45^{R}	0.02	ALLN-HAV	-0.57^{R}	0.00
AVEOST	0.45^{R}	0.20	AVEN-HAV	-0.57^{R}	0.00

Table 5.89: Correlation Of Secondary Osteon And Non-Haversian Canal Counts With Tooth Wear Scores, For The Does (n=42)

Feature Count*	CC	*P*	Feature Count*	CC	*P*
OST1	0.58^{R}	0.00	N-HAV1	-0.81^{R}	0.00
OST2	0.55^{R}	0.00	N-HAV2	-0.75^{S}	0.00
OST3	0.71^{R}	0.00	N-HAV3	-0.75^{R}	0.00
OST4	0.64^{R}	0.00	N-HAV4	-0.76^{S}	0.00
ALLOST	0.70^{R}	0.00	ALLN-HAV	-0.83^{R}	0.00
AVEOST	0.70^{R}	0.00	AVEN-HAV	-0.84^{R}	0.00

<u>Key To Tables 5.80; 5.81; 5.82</u>

*see Figure 5.1

CC - correlation coefficient

P - significance of the correlation coefficient

^{R} - Pearson's correlation (linear)

^{S} - Spearman's rank correlation (non-parametric)

Non-parametric Spearman's rank correlation or linear Pearson's correlation was used as appropriate.

5.7.1 THE WHOLE SAMPLE

Secondary Osteon Counts

- These provided highly significant linear correlations with tooth wear scores.
- All coefficients were positive and ranged from 0.54 (P=0.00) for the count in position 4 (OST4) to 0.65 (P=0.00) for the total and average counts (ALLOST, AVEOST).

Non-Haversian Canal Counts

- These provided highly significant strong correlations with tooth wear scores.
- All counts, except the count in position 4 (N-HAV4), showed a linear correlation with tooth wear score.
- All coefficients were negative and were greater than -0.65 (P=0.00).
- The strongest correlation was provided by the total and average counts (ALLN-HAV, AVEN-HAV) and was -0.79 (P=0.00).

5.7.2 THE BUCKS

Secondary Osteon Counts

- All counts, except the position 3 count (OST3), showed a linear correlation with tooth wear score.
- Correlations were positive and weak, and ranged from 0.25 (P=0.20), for the position 4 count (OST4), to 0.50 (P=0.00), for the position 1 count (OST1).

Non-Haversian Canal Counts

- All counts, except the position 4 count (N-HAV4), showed a linear correlation with tooth wear score.
- All correlation coefficients were negative and weak, and ranged from -0.34 (P=0.07), for the position 4 count (N-HAV4), to -0.59 (P-0.00), for the position 2 count (N-HAV2).

5.7.3 The Does

Secondary Osteon Counts

- All counts showed positive, significant, weak to strong degrees of linear association with tooth wear scores.
- Correlation coefficients ranged from 0.55 (P=0.00), for the position 2 count (OST2), to 0.71 (P=0.00), for the position 3 count.

Non-Haversian Canal Counts

- These provided highly significant strong correlations with tooth wear scores.
- All counts, except the position 2 (N-HAV2) and position 4 (N-HAV4) counts, were linearly correlated with tooth wear scores.
- All correlation coefficients were negative and were >0.74 (P=0.00).

5.7.4 In Summary

- All counts showed highly significant degrees of association with tooth wear scores.
- Non-Haversian canals were negatively correlated with tooth wear scores, and secondary osteon counts were positively correlated with tooth wear scores.
- For the whole sample and the bucks and does separately, non-Haversian canal counts showed stronger degrees of association with tooth wear scores than secondary osteon counts.
- Secondary osteon counts for bucks showed the least degree of association with tooth wear scores.
- Non-Haversian canal counts for the does showed the strongest degree of association with tooth wear scores.

5.7.5 Discussion

Tooth wear scores are known to increase with increasing age (above). Thus the same pattern of correlation as shown between bone histology counts and cement layer ages was expected to be shown between bone histology counts and tooth wear scores, and this was the case. The least degree of association between features, here, was shown for buck bone histology counts and tooth wear scores, earlier it was lowest for buck bone histology counts and cement layer ages. The strongest, most significant degreee of association was between doe bone histology counts and tooth wear scores, and earlier it was greatest between doe bone histology counts and cement layer ages. Finally, levels of association with tooth wear scores were stronger for non-Haversian canal counts than for secondary osteon counts. This echoed the earlier finding that levels of association with cement layer ages were stronger for non-Haversian canal counts than for secondary osteon counts.

5.7.6 In Conclusion

A similar pattern of association between bone histology counts and cement layer ages and between bone histology counts and tooth wear scores were noted.

5.8 The Application Of Histology-Based Ageing To Archaeological Specimens

The histology-based cement layer age predicting regression equations (above) were used to provide cement layer ages for six unknown archaeological roe deer and one modern specimen. Five mandibles were kindly supplied by Alan Pipe of the Museum of London Archaeological Services (MOLAS), and a further two mandibles were supplied by Naomi Mott of the Institute of Archaeology (IOA):

The MOLAS Specimens

- ILA 79 524 - A right mandible with all permanent premolars and molars intact. It was recovered from brown silt near the threshold of a large timber building, dating to the 3rd century A.D., at Miles Lane, London.
- ILA 79 614 - A left mandible with all permanent premolars and molars, but the crown of the first molar was broken. It was recovered from a deliberately infilled drain of silty sand, dating to the 2nd century A.D. and lying on the west side of the building were ILA 79 524 was recovered.
- WP 83 576 - A right mandible with all permanent premolars and molars, from the Roman site of Winchester Palace, Southwark.

Table 5.90: Histology Based, Cement Layer And Tooth Wear Score Inferred Cement Layer Ages For 4 Unknown Roe Deer.

AGE ESTIMATE	IOA 1	IOA 2	ILA 79 614	ILA 79 524
CEMENT LAYER	n/a	n/a	4.00	4.00
Tooth Wear Score	4.84	0.19	2.89	2.48
OST1*	4.39	-4.14	17.9	6.95
√OST2*	10.54	-2.64	10.54	6.71
√OST3*	13.40	8.60	8.45	3.30
OST4*	5.60	3.53	4.12	-1.20
√ALLOST*	9.64	2.76	9.76	4.51
AVEOST*	9.60	1.09	9.78	2.81
√N-HAV1*	4.24	-9.78	-9.38	-6.39
√N-HAV2*	2.36	-13.52	-9.82	-11.59
√N-HAV3*	-5.80	-14.56	-12.44	-10.79
√N-HAV4*	0.05	-11.59	-7.80	-7.32
√ALLN-HAV*	0.01	-12.85	-10.43	-9.40
√AVEN-HAV*	-0.03	-12.80	-10.40	-9.38

n/a = not available

* see Figure 5.1

- WP 83 1110 - A left mandible with all permanent premolars and molars, from the Roman site of Winchester Palace, Southwark.
- CB 81 902 - A right mandible with all permanent premolars and molars, from a yellow clay silt layer at Calverts Building, North Southwark. This particular area of the site dated to pre 1150 A. D.

The IOA Specimens

- IOA 1 - An archaeological left mandible with the third molar missing. The mandible was retrieved from a waterlogged burial environment.
- IOA 2 - A modern right mandible with all premolars and molars intact. The third molar was still in the process of eruption which suggested that the mandible belonged to an animal in its 1st year of life.

Sections were removed from each mandible and prepared and analysed as described in Chapter 4. Four specimens provided sufficient microstructural clarity for histological counts to be made (IOA 1, IOA 2, ILA 79 614 and ILA 79 524). The others showed severe diagnetic alteration, and the histological structure of the bone could not be discerned or quantified. As the sex of the specimens were unknown, reversed regression equations for predicting age derived from regression analysis of secondary and non-Haversian canal counts on cement layer age for the whole sample were used to estimate the age of each specimen. First molars were also removed from each molar and aged using the cement layer technique of Ratcliffe & Mayle (Chapter 1). Finally, tooth wear scores were provided for each mandible by Richard Carter (1996) and these were also inserted into the appropriate reversed regression equation for predicting age. All estimated ages are present in Table 5.90.

5.8.1 IN SUMMARY

Generally, there was poor agreement between the ages estimated through the varying techniques, and several of the bone histology-based estimates were negative:

- For IOA 1 cement layer age could not be assessed as there was no clear layering with the relatively thick pad of cement, tooth wear score age was 4.84 years and histology-based ages ranged from -5.80 to 13.40 years.
- For IOA 2 cement layer age could not be calculated, tooth wear score age was 0.19 years and histology-based ages ranged from -13.52 to 8.60 years.
- For ILA 79 614 cement layer age was 4 years, tooth wear score age was 2.89 years and histology-based ages ranged from -12.44 to 10.54 years.
- For ILA 79 524 cement layer age was 4 years, tooth wear score age was 2.48 years and histology-based ages ranged from -11.59 to 6.95 years.

5.8.2 DISCUSSION

Difficulty was experienced in obtaining cement layer ages. Clarity of layers was extremely poor, and those specimens that did show layering required polishing twice before a count could be made. Even then, confusion was caused by the presence of fine accessory bands within the broader white bands. Such accessory bands have been attributed to disruptions in growth during the summer rut (Chapter 1), but, as the sex of the specimens considered was unknown, it was not known if this was the case here. Two specimens, IOA 1 and IOA 2, did not show layering within their cement covering. One animal (IOA 2) was in its first year of life, as indicated by the partially erupted third molar, and, although there was some cement present, the first white band (which in roe deer forms in their second summer) had not yet begun to develop. The other animal (IOA 1) had been retrieved from a waterlogged burial environment and the cement pad flaked and began to break away from the specimen during preparation, but even in the remaining cement broad white layers could not be seen.

Two specimens, IOA 1 and ILA 79 614, had broken or missing molars and so tooth wear scores had to be estimated from assessments of wear on adjacent teeth. Only one age estimate based on tooth wear score produced an age estimate that agreed with a cement layer estimate. This was for IOA 2, the specimen believed to be in its 1st year of life.

For those specimens that showed sufficient microstructural clarity for histological counts to be made, the counts were conducted with ease. However the ranges of resulting age estimates were large, many ages were negative and there was poor agreement with tooth wear and cement layer ages.

5.8.3 IN CONCLUSION

Problems were encountered with all three ageing techniques. The lack of consistency between age estimates suggests that there is much room for improvement and refinement in the age estimation of roe deer. As the true ages of the above specimens are not known, the performance or accuracy of one ageing technique over another cannot be assessed, but negative histology-based estimates are not acceptable.

CHAPTER 6 CONCLUSIONS

This is the first attempt to apply bone histology-based ageing to a non-human mammalian species that is prevalent in the archaeological record. Bone histology is difficult work and it is hard to define methods for recording features repeatably. The production of thin sections of cortical bone for microscopic analysis was found to be extremely time consuming and fraught with difficulties. Consequently, polished block faces of bone were prepared and analysed in a scanning electron microscope (SEM) in back-scattered electron detector mode. This method of sample preparation was preferred and is recommended, as it can be conducted with comparative ease and speed, and its success is guaranteed. Photographs of each bone field, as they appeared in the SEM, were taken and histological counts were conducted directly from the photographs. As in Kerley (1965), an attempt was made to record four histological features in each bone field: Non-Haversian canals, secondary osteons and circumferential lamellae could be identified and recorded consistently and easily, but fragmentary osteons were difficult to isolate and counts could not be conducted confidently. Others have also have expressed difficulty in counting fragmentary osteons (Ahlqvist & Damsten, 1969; Singh & Gunberg, 1970; Aiello & Molleson, 1993) and, as other features are more easily recorded, their exclusion is recommended. The photographs served as a permanent record of each bone field and facilitated a study of intra- and inter-observer error. This study showed that the adopted recording procedure was very reliable, and that individual counts could be recorded consistently by both the same observer and by different observers. It is felt that one of the achievements of this study was the development of a recording technique which could be applied routinely to a range of archaeological material.

Statistical analysis of the relationships between bone histology counts and cement layer age revealed that circumferential lamellae area showed no significant degree of correlation with, and could not be used to predict cement layer age in roe deer. As periosteal circumferential lamellae rarely survive in archaeological contexts, their lack of association with cement layer age was not considered a problem. Again, it is recommended that in future studies this feature should be excluded from consideration. The strongest correlation of a feature count with cement layer age was provided for the does by both transformed total and transformed average non-Haversian canal counts, and these counts also provided the most accurate cement layer age estimates, although they did tend to underpredict age slightly (Table 6.1). It is difficult to make comparisons with previous studies using alternative methods of age estimation (Aitken, 1975; Bosold ,1968) because they did not investigate the accuracy directly. However, the levels of association with cement layer age and the accuracy of age prediction found in the present study did not compare well to those produced by tooth wear scores on the same material by Richard Carter (1996), for either does or bucks or both combined (Table 6.1). Nonetheless, neither method has a high level of accuracy and, by these standards the best bone histology-based estimates are only slightly worse than those produced by tooth wear scores.

Table 6.1: The Comparative Accuracy of Non-Haversian Canal Counts And Tooth Wear Scores For Predicting Cement Layer Ages (in years)

Age Estimate	Pearson's r	Average Inaccuracy	Average Bias
Doe, Transformed Total Non-Haversian Canal Count (n=42)	-0.75 (*P*=0.00)	2.53	-0.39
Doe, Transformed Average Non-Haversian Canal Count (n=42)	-0.75 (*P*=0.00)	2.50	-0.04
Whole Sample, Tooth Wear Score (n=72)	0.88 (*P*=0.00)**	1.31	0.16
Bucks, Tooth Wear Score (n=30)	0.84 (*P*=0.00)	1.36	0.00
Doe, Tooth Wear Score (n=42)	0.84 (*P*=0.00)	1.66	0.00

** Pearson's r was calculated for the relationship between tooth wear score and transformed (square root of) cement layer age.

Hence, if the cheek teeth are recovered intact, the tooth wear score age estimation technique is preferred over the histology-based age estimation technique because:

- Tooth wear scores have been shown to produce more accurate estimations of age than histological counts.

- Unlike histological counts, tooth wear scores do not appear to be affected by the sex of the specimen. This is a clear advantage in archaeology, where information regarding gender may not be available.
- Tooth wear scores can be obtained without damaging specimens and without access to laboratory equipment, such as a scanning electron microscope.

However, it should be noted that when age estimates based on tooth wear scores were compared with cement layer ages in two archaeological roe deer there was poor agreement between estimates. For both specimens tooth wear scores underpredicted cement layer age by >1 year, and did not overpredict them by 0.16 years as previous analysis (above) had suggested should be the case.

Bone histology-based ageing does, however, have a role where the teeth are poorly preserved and in spite of the difficulties encountered, the work carried out in this study does suggest some potential for future developments. It is suggested that such developments could concentrate on the following lines:

- A sample of known age material, tagged at birth and with cull date known, should form the main focus of the study. This would require considerable effort.
- Age estimates based on tooth wear scores, cement layer counts and bone histology should all be assessed on the same body of material.
- The scanning electron microscope based methodology developed here should be used in preference to thin section and light microscope methods.
- Bone histology counts could be restricted to non-Haversian canals and secondary osteons.
- More detailed consideration of the inner regions of the cortex is likely to yield a better return than the current more generalised study, which included analysis of the outer cortical region.

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HISTOLOGICAL COUNTS

Original Counts for Roe Bucks

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C3/84	0	0	1	0	1	0.25	0	0	0	0	0	0	121	132	162	175	590	147.50
C3/85	0	2	0	47	49	12.25	0	0	0	0	0	0	132	225	219	81	657	164.25
C8/84	0	0	0	0	0	0	0	0	0	0	0	0	108	170	148	109	535	133.75
C10/84	9	5	0	3	17	4.25	0	0	0	0	0	0	60	123	183	138	504	126
CGC13/83	32	21	45	22	120	30	0	0	0	0	0	0	152	135	114	68	469	117.25
C30/85	27	21	8	20	76	19	0	0	0	0	0	0	43	79	120	71	313	78.25
C11/84	31	71	90	72	264	66	8	0	0	5	13	3.25	43	32	16	39	130	32.50
C9/85	40	23	10	12	85	21.25	5	0	0	2	7	1.75	51	158	198	75	482	120.50
CGC5/83	48	73	28	67	216	54	0	0	0	4	4	1	5	16	99	5	125	31.25
CGC9/83	37	48	27	57	169	42.25	13	0	0	0	13	3.25	32	51	154	29	266	66.50
C25/85	5	0	0	4	9	2.25	0	0	0	0	0	0	116	201	210	168	695	173.75
CGC25/83	7	15	0	37	59	14.75	0	0	0	0	0	0	95	103	153	52	403	100.75
C15/84	22	52	47	0	121	30.25	3	0	0	0	3	0.75	58	74	88	0	220	55
C10/85	47	62	15	27	151	37.75	22	0	0	6	28	7	24	55	154	32	265	66.25
CGC10/83	52	50	15	53	170	42.50	0	0	0	0	0	0	13	27	119	0	159	39.75
C26/84	15	17	0	13	45	11.25	7	0	0	0	7	1.75	73	141	193	64	471	117.75
C18/85	5	4	2	9	20	5	8	0	1	1	10	2.50	72	148	147	52	419	104.75
C16/84	43	28	3	17	91	22.75	6	0	0	0	6	1.50	29	77	20	105	231	57.75
C23/84	25	16	2	2	45	11.25	0	0	0	0	0	0	42	109	165	150	466	116.50
CGC20/83	11	3	0	15	29	7.25	0	0	1	0	1	0.25	64	179	175	116	534	133.50
C22/84	27	48	22	40	137	34.25	0	0	0	0	0	0	44	7	102	18	171	42.75
C21/85	76	0	1	27	104	51	28	0	0	0	28	7	15	110	169	66	360	90
C23/85	0	0	0	0	0	0	0	0	0	0	0	0	157	139	179	184	659	164.75
C9/84	21	34	22	0	77	19.25	1	1	0	0	2	0.50	70	24	156	0	250	62.50
C14/84	43	25	14	5	87	21.75	0	0	0	0	0	0	89	167	118	188	562	140.50
C17/84	45	77	30	61	213	53.25	0	0	20	5	25	6.25	15	3	64	4	86	21.5
C20/84	14	9	0	23	46	11.50	16	0	0	0	16	4	61	110	167	80	418	104.50
CGC11/83	24	26	27	27	104	26	0	0	0	0	0	0	51	63	52	62	228	57
C27/84	54	49	47	46	196	49	0	0	4	4	8	2	0	8	60	16	84	21
C30/84	48	50	53	47	198	495	0	0	0	0	0	0	14	21	34	8	77	19.25

Original Counts for Roe Does

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C10/85	0	0	0	0	0	0	0	0	0	0	0	0	174	190	201	184	749	187.25
C51/54/84	3	0	0	0	3	0.75	0	0	0	0	0	0	133	168	201	173	675	168.75
C21/84	38	85	18	40	181	45.25	0	0	0	0	0	0	44	12	163	54	273	68.25
C7/85	0	0	0	28	28	7	0	0	0	0	10	2.50	0	0	219	69	288	72
C27/85	21	2	2	2	27	6.75	3	0	0	3	6	1.50	77	204	175	98	554	138.50
C32/84	4	0	2	1	7	1.75	0	0	0	0	0	0	124	223	216	11	574	143.50
C64/84	20	45	25	41	131	32.75	5	0	0	0	5	1.25	118	55	68	47	288	72
C49/84	5	17	0	37	59	14.75	2	0	0	6	8	2	124	129	185	47	485	121.25
C55/84	13	6	8	17	44	11	0	0	0	0	0	0	10	49	85	15	159	39.75
C47/85	0	7	2	2	11	2.75	0	0	0	0	0	0	153	150	171	131	605	151.25
C22/85	14	3	14	24	55	13.75	0	0	4	0	4	1	144	208	125	81	558	139.50
C5/85	50	63	51	50	214	53.50	1.5	0	0	0	1.5	0.38	16	10	9	4	39	9.75
C3/85	25	37	18	16	96	24	0	0	0	0	0	0	65	95	142	59	361	90.25
C56/84	69	98	86	69	322	80.50	0	0	0	8	8	2	18	42	45	43	148	37
C40/85	38	30	8	35	111	27.75	3	0	0	3	6	1.50	38	44	179	40	301	72.25
C29/83	61	82	64	34	241	60.25	0	0	0	0	0	0	24	0	24	41	89	22.25
C1/85	62	101	59	44	266	66.50	0	0	9	1	10	2.50	23	13	80	45	161	40.25
C53/84	42	66	24	43	175	43.75	0	0	0	0	0	0	35	14	120	36	205	51.25
CX/84	52	56	39	62	209	52.25	14	0	0	6	20	5	12	24	87	6	129	32.25
C28/83	22	5	24	40	91	22.75	5	3	26	2	36	9	63	111	83	21	278	69.50
C7/83	38	57	65	49	209	52.25	0	0	0	0	0	0	37	23	27	15	102	25.50
C59/84	47	28	22	71	168	42	6	0	0	0	6	1.5	52	135	140	25	352	88
C18/83	61	56	39	14	170	42.50	0	0	0	0	0	0	12	8	51	84	155	38.75
C4/84	8	4	36	49	97	24.25	17	11	2	6	36	9	40	30	26	30	126	31.50
C1/84	58	56	52	43	209	52.25	0	0	0	0	0	0	0	29	15	16	60	15
C28/85	101	55	85	54	295	73.75	0	2	0	1	3	0.75	0	16	5	14	35	8.75
CN/84	39	37	29	62	167	41.75	5	1	2	2	10	2.5	24	90	88	40	242	60.50
C48/85	44	38	54	62	198	49.50	2	0	0	0	2	0.50	12	10	33	5	60	15
CZ/84	30	41	61	23	155	38.75	0	0	0	0	0	0	43	27	12	48	110	27.50
C9/83	60	51	37	71	219	54.75	0	0	0	0	0	0	30	58	66	28	182	45.50
CA/84	15	51	34	50	149	37.25	0	0	0	0	0	0	31	13	58	8	110	27.5
CD/84	52	94	63	41	250	62.50	16	0	0	1	17	4.25	3	10	20	7	40	10

Original Counts for Roe Does (cont'd)

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C7/84	31	90	46	47	214	53.50	6	0	4	3	13	2.25	30	29	59	10	128	32
C33/85	61	68	51	76	256	64	0	0	0	1	1	0.25	0	0	30	2	32	8
C20/83	19	32	15	24	90	22.50	0	0	0	0	0	0	16	72	120	39	247	61.75
CK/84	26	40	18	48	132	33	3	0	0	0	3	0.75	38	43	90	30	201	50.25
CM/84	36	0	67	45	148	37	0	0	0	0	0	0	12	0	4	8	24	6
C18/84	49	77	68	57	251	62.75	0	0	0	0	0	0	4	11	13	12	40	4
C32/85	65	97	64	83	309	77.25	0	0	0	0	0	0	6	10	22	7	45	11.25
CU/84	62	76	70	33	241	60.25	0	0	0	0	0	0	0	1	5	5	11	2.75
CAB/8453	53	67	50	61	231	57.75	0	0	0	0	0	0	6	9	19	4	38	9.50
C9/85	0	0	0	0	0	0	0	0	0	0	0	0	163	129	118	101	511	127.75

Recounts for Roe Bucks

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C3/84	0	0	1	0	1	0.25	0	0	0	0	0	0	123	132	159	175	589	147.25
C3/85	0	2	0	47	49	12.25	0	0	0	0	0	0	132	221	219	81	653	163.25
CGC13/83	31	21	45	22	119	29.75	0	0	0	0	0	0	154	136	114	68	472	118
C11/84	29	67	92	72	260	65	7	0	0	5	13	3.25	43	31	16	39	129	32.25
CGC5/83	48	71	28	67	214	53.50	0	0	0	4	4	1	5	16	99	5	125	31.25
C25/85	5	0	0	4	9	2.25	0	0	0	0	0	0	112	200	202	165	679	169.75
C15/84	22	47	47	0	116	29	3	0	0	0	3	0.75	58	67	87	0	212	53
C10/85	47	62	13	27	149	37.25	20	0	0	6	26	11.50	24	55	152	32	263	65.75
C18/85	5	4	2	9	20	5	8	0	1	1	10	2.50	72	148	145	52	417	104.25
C22/84	27	47	22	40	136	34	0	0	0	0	0	0	44	7	100	18	169	42.25
C21/85	74	0	1	27	102	25.50	26	0	0	0	26	6.50	15	108	170	67	360	90
C23/85	0	0	0	0	0	0	0	0	0	0	0	0	152	141	178	187	658	164.50
C14/84	43	25	14	5	87	21.75	0	0	0	0	0	0	89	167	117	191	564	141
CGC11/83	24	26	25	26	101	25.25	0	0	0	0	0	0	51	62	52	62	227	56.75
C27/84	54	49	49	51	203	50.75	0	0	4	4	8	2	0	8	60	16	84	21

Recounts for Roe Does

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C10/85	0	0	0	0	0	0	0	0	0	0	0	0	173	191	199	184	747	186.75
C27/85	21	2	2	2	27	6.75	3	0	0	3	6	1.50	77	202	175	98	552	138
C49/84	5	17	0	36	58	14.50	2	0	0	6	8	2	123	129	180	46	478	119.50
C47/85	0	7	2	2	11	2.75	0	0	0	0	0	0	151	150	169	131	601	150.25
C5/85	49	63	51	50	213	53.25	1	0	0	0	1	0.25	16	10	9	4	39	9.75
C40/85	38	30	8	34	110	27.50	3	0	0	3	6	1.50	38	41	177	40	296	74
C29/83	61	79	67	34	241	60.25	0	0	0	0	0	0	24	0	24	41	89	22.25
C28/83	22	5	24	41	92	23	5	3	26	2	36	9	63	114	83	21	281	70.25
C7/83	38	55	63	49	205	51.25	0	0	0	0	0	0	37	23	27	16	103	25.75
C4/84	8	4	34	47	93	23.25	18	12	2	7	39	9.75	41	30	26	30	127	31.75
C1/84	57	56	52	43	208	52	0	0	0	0	0	0	0	28	15	16	59	14.75
CN/84	39	37	29	62	167	41.75	5	1	2	2	10	2.50	24	93	88	40	245	61.25
C48/85	44	38	52	62	196	49	2	0	0	0	0	0	12	10	33	5	60	15
C9/83	64	56	39	60	219	54.75	0	0	0	0	0	0	35	52	66	32	185	46.25
CD/84	51	89	61	41	242	60.50	14	0	0	1	15	3.75	3	10	20	7	40	10
C7/84	31	87	47	47	212	53	6	0	4	3	13	3.25	30	29	55	10	124	31
C20/83	19	32	15	24	90	22.50	0	0	0	0	0	0	16	74	118	39	247	61.75
C18/84	49	75	64	54	242	60.50	0	0	0	0	0	0	4	11	13	12	40	10
C32/85	61	92	64	84	301	75.25	0	0	0	0	0	0	6	10	22	7	45	11.25
CU/84	63	75	74	37	249	62.25	0	0	0	0	0	0	0	1	5	5	11	2.75
CAB/84	53	61	49	62	225	56.25	0	0	0	0	0	0	6	9	19	4	38	9.50

Observer B's Roe Buck Counts

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C3/84	0	0	1	0	1	0.25	0	0	0	0	0	0	117	129	161	176	583	145.75
CGC5/83	48	71	28	68	215	53.75	0	0	0	3	3	0.75	5	16	100	5	126	31.5
C18/85	5	4	2	8	19	4.75	7	0	1	2	10	2.50	72	141	144	52	409	102.25
C14/84	40	24	12	5	81	20.25	0	0	0	0	0	0	87	160	111	190	548	137
C27/84	54	47	52	52	205	51.25	0	0	5	4	9	2.25	0	8	54	16	78	19.50

Observer B's Roe Doe Counts

SPECIMEN	OST1	OST2	OST3	OST4	ALLOST	AVEOST	CIRC1	CIRC2	CIRC3	CIRC4	ALLCIRC	AVECIRC	N-HAV1	N-HAV2	N-HAV3	N-HAV4	ALLN-HAV	AVEN-HAV
C27/85	18	2	2	2	24	6	3	0	0	3	6	1.50	69	195	174	93	531	132.75
C5/85	47	61	50	46	204	51	2	0	0	0	2	0.50	14	10	10	4	38	9.50
C28/83	19	5	23	37	84	21	5	4	23	2	34	8.50	59	105	79	21	264	66
CN/84	37	37	26	27	127	31.75	5	1	2	2	10	2.5	25	91	85	40	241	60.25
CU/84	56	70	70	42	238	59.50	0	0	0	0	0	0	0	1	4	5	10	2.25